NEURAL CONTROL OF ADRENOCORTICAL GROWTH

by

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ABSTRACT

Changes in adrenal wet and dry weight, plasma corticosterone and ACTH were measured in rats at various times after unilateral adrenalectomy or sham unilateral adrenalectomy. A relative hypocorticoid signal and hypersecretion of ACTH were observed only within the initial 2 hours after unilateral adrenalectomy. The weight of the remaining adrenal was significantly increased by 12 hours and increased further at 72 hours and 10 days. As well as in intact rats, compensatory adrenal growth occurred in hypophysectomized animals and in rats treated with dexamethasone suggesting that ACTH and other pituitary hormones were not required for the compensatory growth response. The increased adrenal weight after unilateral adrenalectomy consisted of increases in DNA content followed by increases in RNA and protein content; the growth response was characterized as compensatory adrenal hyperplasia, not compensatory adrenal hypertrophy. Treatment with ACTH decreased the increases in DNA content after unilateral adrenalectomy; and removal of circulating ACTH by dexamethasone treatment accelerated the development of compensatory adrenal growth. Unilateral hypothalamic lesions in the dorsolateral ventromedial nucleus or in the ventral premammillary and lateral mammillary nuclei blocked compensatory adrenal growth when placed ipsilateral to the side of subsequent adrenalectomy; lesions placed in similar sites contralateral to the side of adrenalectomy had no inhibitory effects. Spinal cord hemi-sections at thoracic level -2 inhibited the growth response when placed contralateral to the side of subsequent adrenalectomy; ipsilateral hemi-sections at the same spinal cord level had no inhibitory effects. Finally, treatment of

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the adrenal gland and pedicle with a local anesthetic prior to removal of the treated adrenal blocked compensatory growth of the remaining adrenal. Together, these experiments suggest that afferent and efferent neural components mediate the response to unilateral adrenalectomy. Additional experiments showed that manipulation of one adrenal stimulated weight increases in the other adrenal at 12 hours. The weight increases, consisting of DNA, RNA and protein content increases, were similar to changes observed after unilateral adrenalectomy. Adrenal dry weight was also elevated after unilateral adrenalectomy or manipulation, and there was no difference in residual blood per gland as measured by the ^{51}Cr tagged red cell content. The observation that adrenal growth could be stimulated unilaterally in the presence of both adrenals supported the hypothesis that nerves were involved in the compensatory growth response. In summary, these studies demonstrate that compensatory adrenal growth is mediated by a reflex composed of afferent and efferent neural components and is probably integrated within the hypothalamus.

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CHAPTER I

INTRODUCTION

Overview of hypothalamo-pituitary-adrenal axis

The functional characteristics of the hypothalamo-pituitary-adrenal axis have been outlined by the work of many investigators over the past fifty years. An overview of this work will attempt to summarize our current knowledge of the adrenocortical system. The initial work of Smith (1930) which showed that the rat adrenal gland atrophied following removal of the pituitary gland and that the atrophy could be prevented by replacement with anterior pituitary extract suggested that a factor or factors released from the anterior pituitary normally maintained the morphological integrity of the adrenal gland. These experiments also showed that the adrenal medulla was not greatly affected by hypophysectomy and therefore, that the pituitary factor influenced primarily the adrenal cortex. Work reporting the isolation of an acetone-soluble pituitary fraction which in small amounts caused 50-200% increases in adrenal weight without influencing other endocrine tissues (Collip, Anderson and Thomson, 1933), was followed by the identification and standardization of sheep adrenocorticotrophic hormone (ACTH) through bioassay in hypophysectomized rats (Simpson, Evans, and Li, 1943); the chemical structure of ACTH as a polypeptide of 39 amino acids was elucidated 11 years later (Bell, 1954).

With the availability of synthetic ACTH and the purification of the native molecule, characterization of the effects of ACTH on the adrenal cortex was made possible. While all three zones of the adrenal cortex are stimulated by ACTH, the predominant effects are on the zona fasciculata and zona reticularis. The zona glomerulosa, unlike the zona fasciculata and reticularis, is also stimulated by non-pituitary factors (e.g. angiotensin II) (Davis, 1975). The ACTH molecule stimulates both secretion from, and growth of the adrenal cortex. Increases in adrenal venous output of glucocorticoids are observed following the administration of ACTH (Nelson and Hume, 1955; Urquhart and Li, 1968). A number of steps in the steroidogenic pathway are stimulated by ACTH (Gill and Garren, 1973), and removal of the hormone through hypophysectomy leads to a nearly complete disruption of the steroid-secreting capabilities of the gland (Liddle, Island and Meador, 1962). As a growth-promoter, ACTH initially causes cell hypertrophy by increasing RNA and protein content and following a longer latency, it induces adrenal hyperplasia by increasing DNA synthesis leading to cell proliferation (Fiala, Sproul and Fiala, 1956; Imrie, et al., 1971). In summary, ACTH released from the pituitary maintains and promotes the secretory function and growth of the adrenal cortex.

While the effects of pituitary hormones on peripheral tissues were being elucidated, Harris (1948) was considering the influence of the brain on the function of the pituitary. His investigations showed that transplantation of the pituitary to the temporal lobe of the cerebral cortex or to the anterior chamber of the eye resulted in adrenocortical atrophy; however, if the pituitary was transplanted under the median eminence of the hypothalamus and the hypophyseal portal system regrew, the adrenal cortex would not atrophy (Harris and Jacobsohn, 1952). These experiments showed that the integrity of the portal vessels was essential for the maintenance of adrenocortical function and suggested that a humoral substance released from the hypothalamus could stimulate the pituitary synthesis and secretion of ACTH. This hypothesis was later supported by the preparation of hypothalamic extracts which stimulated ACTH release (Leeman, Glenister and Yates, 1962). Thus, secretion from the hypothalamus of a corticotropic-releasing factor (CRF), believed to be synthesized and released from neurons (Edwardson and Bennett, 1974), stimulates the synthesis and secretion of ACTH which is transported in plasma to the adrenal cortex to stimulate steroidogenesis and maintain glandular viability.

Earlier studies had shown that increases in plasma levels of adrenal corticoids inhibited production of ACTH (Ingle, Higgins and Kendall, 1938) and that decreases in adrenal corticoids resulted in stimulation of ACTH synthesis (Sayers and Sayers, 1947; Taylor, Albert and Sprague, 1949). In more recent work, the concept of "negative feedback" of adrenal corticoids has been confirmed and found to occur at the pituitary (Russell, et al., 1969; Sayers and Portonova, 1974) and at the hypothalamus (Endroczi, Lissak and Tekeres, 1961; Sato, et al., 1975). Increased secretion of CRF leading to higher plasma concentrations of ACTH causes an increase in plasma corticoids; the increased levels of corticoids feedback at the hypothalamus to inhibit CRF and at the pituitary to decrease ACTH secretion directly.

The adrenocortical system is activated by input to the CRF neuron from at least two sources. First, trauma produced by various stimuli can cause increased synthesis and release of CRF (Hiroshige, Abe and

Sato, 1971; Sato *et al.*, 1975). Second, the adrenocortical system is characterized by a prominent diurnal or circadian rhythm in the secretion of ACTH and glucocorticoids (Nichols and Tyler, 1967; Orth and Island, 1969). While the origin of the stimulus to this rhythm is unknown, the finding that CRF levels in the hypothalamus also demonstrate a circadian rhythm (Hiroshige and Sakakura, 1971) suggests that the controlling mechanism acts through the CRF neuron.

Evidence for involvement of the autonomic nervous system in adrenocortical function

Study of the role of the nervous system in regulating the adrenocortical system has been restricted primarily to identifying the central neural structures involved in stress-induced activation of CRF (Porter, 1953; Seggie, Uhlir and Brown, 1974), feedback control of CRF (Feldman and Dafny, 1970; Allen and Allen, 1975), or initiation of the diurnal rhythm (Moberg, *et al.*, 1971; Campbell and Ramaley, 1974). While it is well established that the sympathetic nervous system controls the secretion of adrenal medullary catecholamines through direct cholinergic innervation of chromaffin cells, little is known about the role of the autonomic nervous system in adrenocortical function. Early histological examination of the adrenal gland at the light microscopic level indicated that the cortex and capsular elements received no direct innervation; all fibers in the cortex were observed to end on blood vessels or to transverse the cortex to terminate on medullary cells (Hollinshead, 1936; Swinyard, 1937; MacFarland and Davenport, 1941). These reports, however, were contradicted by additional light microscopic studies which showed dense innervation of all three cortical zones in addition to nerve fibers passing to the medulla (Albert, 1931; Mikhail, 1961; Mikhail and Amin, 1969). The differing conclusions of these authors concerning cortical innervation may have been due to the non-specificity of the silver staining techniques used; at the light microscopic level using these stains, connective tissue strands and nerve fibers can sometimes be confused. Working at the ultrastructural level, this problem can be circumvented. Recently, Unsicker (1969, 1971) and Alvarez (1970) working at the electron microscopic level showed in a number of species, including man, that nerve endings come in close contact with endocrine cells of all three cortical zones. These "contacts" were identified histologically as synapses since presynaptic vesicles and 200Å synaptic clefts were present; however, no postsynaptic specializations were observed. Endings without vesicles adjacent to endocrine cells were also noted by one of these authors (Unsicker, 1971) who suggested that these might represent morphological analogs of sensory receptors.

There is some evidence for the presence of sensory nerve fibers in the adrenal cortex. Kiss (1951) using an axonal degeneration technique found the adrenal cortex of dogs to be supplied by afferents from spinal ganglia at thoracic level 9-11. Neurophysiological evidence for adrenal afferents has also been reported; Niijima and Winter (1968a, b) observed afferent discharges in dissected filaments of nerves from the adrenal gland in the rabbit and cat. In some fibers spontaneous firing could be increased by increasing systemic blood pressure; in other, spontaneously firing, fibers, whereas changes in blood pressure did not alter firing rate, intravenous injections of catecholamines or acetylcholine resulted in cessation or depression of the firing rate. Finally, in normally silent fibers, mechanical

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distention of the adrenal capsule evoked phasic, rapidly adapting trains of impulses. These findings led to the hypothesis that the adrenal gland is supplied by baroreceptors, chemoreceptors and mechanical stretch receptors.

Capsular stretch receptors were the subject of an early paper by Halasz and Szentagothai (1959) who found that unilateral adrenalectomy in the rat resulted in unilateral morphological changes in the cell bodies of the hypothalamic ventromedial nucleus (VMN). Following the removal of one adrenal, nuclei of cell bodies in the VMN contralateral to the side of adrenalectomy hypertrophied while nuclei of cell bodies in the VMN ipsilateral to the side of gland removal atrophied. Bilateral celiac ganglionectomy resulted in hypertrophied nuclei of cells in both VMN. In addition, treatments which increased the size of both adrenal glands such as ACTH administration or chronic stress caused bilateral atrophy of cell nuclei in the VMN; decreasing the size of the gland through corticoid treatment resulted in bilateral hypertrophy of nuclei in the VMN. From these experiments the authors hypothesized that a crossed neural pathway existed from the adrenal gland to the hypothalamus and that adrenal capsular stretch receptors were monitoring gland size; presumably, the hypothalamic changes reflected alterations in afferent neural activity. It was further suggested that the adrenal cortex, and not the adrenal medulla , was being monitored since ACTH and cortisol treatments were known not to have major effects on the adrenal medulla. Similar karyometric analyses were performed in the guinea pig (Sajonski, Smollich and Trolldenier, 1965) and the same unilateral morphological changes were observed in the VMN. Recently, Gerendai, _et al_., (1974)

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have confirmed the hypothalamic changes that occur following unilateral adrenalectomy using autoradiographic techniques at the light and electron microscopic level. Following removal of one adrenal and the injection of labelled amino acid, animals were found to have an increased number of autoradiographic silver grains in the VMN contralateral to the side of adrenalectomy. The unilateral increase in protein-synthesizing activity in the VMN provides additional support for the Halasz and Szentagothai (1959) hypothesis concerning adrenal afferent nerves; however, the functional significance of these neural elements remains obscure.

Two lines of research have been used to investigate the possible involvement of efferent neural elements in adrenocortical function. A number of workers have studied the effects of adrenal denervation on the adrenal response to different stresses. For example, Vogt (1947) made histological comparisons between denervated and innervated adrenals in cats and rats following various stresses. While no difference in lipid content was found between denervated and innervated adrenals in response to cold or heat stress or hemorrhage, the normal lipid depletion following insulin-induced hypoglycemia was prevented by denervation. These studies were followed by a series of experiments in which histological changes in adrenals were observed 17-20 days following denervation (Hata, 1959; Aburaya and Hata, 1961). These experiments showed that the denervated adrenal was atrophied and hypofunctioning compared to the innervated adrenal. In two additional reports comparing the denervated and innervated adrenal in dogs (Yudaev and Pankov, 1961) and in rats (Schapiro, 1963), no difference was found in the secretion of 17-hydroxycorticoids to various stresses. Each

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of the denervation studies can be criticized on the grounds that changes produced by cutting adrenal nerves may have been due to the physical impairment of the adrenal blood supply and that changes in function may have been the result of vascular embarrassment.

To avoid this problem, workers have employed a different approach to study the effects of efferent nerves on the adrenal cortex. Vogt (1944) showed that electrical stimulation of the splanchnic nerve in dogs and cats resulted in increases in adrenal corticoid output. Later work by Okinaka, et al., (1960) reported that the cortisol to corticosterone secretion ratio in dogs changed following splanchnic stimulation. In dogs the output of cortisol is normally equal to the output of corticosterone; following exogenous ACTH administration, both steroids increase equally and the ratio remains constant. However, in response to splanchnic stimulation, the secretion rate of cortisol increased while that of corticosterone decreased. Support for these findings is provided by Hata (1959) and Kitagawa (1959a, b) who suggested that nerves from the hypothalamus may be involved in controlling adrenocortical secretion. These papers reported that electrical stimulation of the posterior hypothalamus in hypophysectomized rats increased the release of glucocorticoids from the adrenal cortex while stimulation of the thalamus and cortex had no stimulatory effect. Unfortunately, the exact location of the stimulation sites could not be obtained from the original reports. Other work implicating hypothalamic efferents to the adrenal cortex showed that unilateral lesions placed in the ventromedial nucleus of the hypothalamus resulted in unilateral histological changes in the adrenal zona fasciculata; the nuclear

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volume of zona fasciculata cells decreased in the adrenal contralateral to the side of hypothalamic lesion (Smollich and Docke, 1969). A crossed, descending pathway was postulated but no function could be ascribed to it. Finally, some *in vitro* evidence to support a role for efferent adrenal nerves has been reported by Rosenfeld (1955) using isolated perfused calf adrenals. Stimulation of the adrenal with acetylcholine increased cortisol production by 20-50%; this cholinergic influence was blocked by atropine but not affected by administration of catecholamines.

Evidence to support the contention that the autonomic nervous system influences adrenocortical function is scattered throughout the literature. However, no attempt has been made to thoroughly and systematically investigate the physiological significance of adrenocortical nerves. The work presented in subsequent chapters of this dissertation was done to examine the influence of adrenal neural elements on the adrenocortical system. Specifically, this report consists of a re-examination of the mechanisms responsible for compensatory adrenal growth after unilateral adrenalectomy.

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CHAPTER II

COMPENSATORY ADRENAL GROWTH IN THE ABSENCE OF CORTICOSTEROIDS AND ACTH

A. INTRODUCTION

Growth of the adrenal cortex can be affected by a variety of hormones. For example, the administration of estrogens or thyroid hormones results in increases in the size of adrenocorticoid cells; the "hypertrophic" action of these factors is probably indirect since removal of the pituitary diminishes their effectiveness (Ingle, 1951). In contrast, androgens and progesterone can cause adrenal atrophy; again, this effect is probably mediated through some pituitary factor (e.g., ACTH). Insulin and growth hormone have been shown to increase adrenal weight by increasing the number of adrenocortical cells. This "hyperplastic" effect appears to result from direct adrenal stimulation by these agents (Ingle, 1951; Bransome and Reddy, 1961). While the physiological significance of these hormones in controlling adrenocortical growth has not been well delineated, it is important to note that factors other than ACTH, directly or indirectly, could influence normal adrenocortical growth.

Nonetheless, ACTH stimulation remains the primary determinant of growth of the adrenal cortex. Early work studying stress-induced activation of the adrenal system used changes in adrenal weight as an index of ACTH stimulation (Fortier, 1959; Dear and Guillemin, 1960). Other changes in the adrenal following ACTH administration have been utilized as a bioassay for ACTH; these include a fall in cholesterol (the precursor molecule for adrenal steroids) (Laqueur, et al., 1955), and a depletion of ascorbic acid (McCann, 1953; Redgate, 1961). The growth-stimulating effects of ACTH

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have been examined in detail by numerous investigators (e.g., Fiala et al., 1956; Bransome and Reddy, 1961; Imrie et al., 1965). The consensus of these reports is that initially ACTH produces rapid increases in cytoplasmic RNA content, followed by increased cytoplasmic and mitochondrial protein synthesis; this early phase which begins within 2 to 3 hours of stimulation has been characterized as a period of cell hypertrophy. The second phase, which begins no earlier than 72 hours, is characterized by increases in DNA synthesis and cell replication. The hyperplastic response occurs following stimulation by large amounts of ACTH; normally, the increased adrenal weight produced by endogenous ACTH stimulation is due to cell hypertrophy, not cell hyperplasia (Bransome and Reddy, 1961).

Early work studying adrenocortical growth in rats found that removal of one adrenal led to dramatic increases in the weight of the remaining adrenal (MacKay and MacKay, 1926). Forty days after unilateral adrenalectomy, the remaining adrenal increased in weight by 61%; this increase which was restricted to the cortex was called "compensatory adrenal hypertrophy". Recent approaches to the study of the phenomenon have been to determine the morphological and biochemical changes that occur in the remaining adrenal following unilateral adrenalectomy. In dogs, Bransome and Reddy (1961) have shown that adrenal DNA concentration doubles by 7 days following unilateral adrenalectomy. The finding of increased DNA concentration in dogs is supported by work in rats showing a 15% increase in cell number by 3 days following unilateral adrenalectomy (Pelligrino, Ricci and Tongiani, 1963). The hyperplastic growth continues until 15 days when the number of cells has increased to 130% of control adrenals; by the 2nd week of growth, cell hypertrophy is also noted. The authors concluded that the response to unilateral adrenalectomy has 3 stages: first, the rate of cell replication increases while cell volume decreases; second, cell volume increases while cell replication rate decreases; and third, cell volume and replication rates return to normal levels. Study of the early phases of adrenal hyperplasia has led to a determination of the adrenal zones where hyperplasia occurs. Using tritiated-thymidine uptake as a measure of DNA synthesis, Reiter and Pizzarello (1966) found that at 2 days following unilateral adrenalectomy in young rats, the highest activity occurred in the outer zona fasciculata. This was subsequently confirmed by Omoto (1969) who noted the build up of new, immature cells at outer regions of the zona fasciculata in rat adrenals 3 days after unilateral adrenalectomy.

Because of the profound effects of ACTH on adrenal growth and the feedback relationship which exists between adrenal steroids and ACTH secretion, a logical hypothesis to explain compensatory adrenal hypertrophy was that removal of one adrenal would produce a hypocorticoid signal which in turn would decrease the feedback brake on ACTH secretion; growth of the remaining adrenal would result from hypersecretion of ACTH. This explanation for compensatory adrenal growth was given initially in the thirties (Collip et al., 1933; Shumacher and Firor, 1934) and was tested using hypophysectomized rats. Removal of one adrenal in hypophysectomized rats did not lead to increases in the weight of the remaining adrenal when compared to adrenals from control animals. This finding supported the contention that increases in ACTH were responsible for compensatory growth. However, closer examination of these experiments shows that the control

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animals used were unilaterally adrenalectomized animals with intact pituitaries; thus, the decreased adrenal weight in the hypophysectomized animal might not be due to removal of the factor responsible for compensatory adrenal hypertrophy but might only reflect the loss of any pituitary factor which effects adrenocortical growth. Although this criticism does not rule out ACTH as the causal factor in compensatory adrenal hypertrophy, it indicates that the early compensatory hypertrophy work did not adequately delineate the mechanisms responsible for the growth phenomenon.

Several lines of evidence do not support the hypothesis that increased secretion of ACTH is solely responsible for compensatory growth. Unilateral adrenalectomy results in an increase of the mitotic index in the cortex of the remaining gland (Carr, 1959). Growth hormone treatment of hypophysectomized rats increases the mitotic index of the adrenal cortices, whereas ACTH treatment of similar animals does not result in an increased mitotic index within the time period studied (Cater and Stack-Dunne, 1955). Compensatory adrenal enlargement was found in hypophysectomized rats that were unilaterally adrenalectomized 11 days previously when the weight of the remaining right adrenal was compared to the right adrenal weight in hypophysectomized rats that were sham adrenalectomized (Lipscomb and Critchlow, 1966).

The hypothesis that low corticoids and secondary increases in ACTH secretion mediate compensatory adrenal growth after unilateral adrenalectomy has never been directly tested to our knowledge. We have tested the hypothesis by measuring plasma ACTH and corticosterone levels and right adrenal weight with time after left adrenalectomy or sham operation in the rat.

B. MATERIALS AND METHODS

Male Sprague-Dawley rats (Simonsen, Gilroy, Ca.) weighing 100-150 g were used. The rats were received 1 to 7 d before experiments and were housed 2 or 3 per cage under controlled lighting (lights on from 0600 h until 1800 h). Rat chow and water were available ad lib. Operations were conducted between 0800 and 1100 h.

Dexamethasone phosphate (Decadron^R, Merck Sharp and Dohme) 25 ug/100 g BW was injected subcutaneously either once or several times as noted in Results. Hypophysectomy was performed by the parapharyngeal approach under ether anesthesia 2-4 hours prior to adrenal surgery. Hypophysectomized rats were given 5% dextrose in water to drink.

Rats were anesthetized with ether or sodium pentobarbital (Nembutal, Abbott) 5 mg/100 g BW, or sodium methohexital (Brevital, Lilly) 5 mg/100 g BW, as indicated in the Results. Left adrenalectomy was performed through a dorsal midline skin incision and a lateral retroperitoneal incision. Left surgical sham adrenalectomy was performed through similar incisions and the gland was observed but not touched. Skin incisions were clipped, and the rats were returned to their home cages until killed.

All rats were killed by decapitation at various times postoperatively and trunk blood was collected into heparinized plastic tubes chilled on ice. The blood was centrifuged at 4°C, and plasma was frozen for subsequent ACTH and corticosterone assays. Adrenals were collected on filter paper saturated with 0.9% NaCl in closed dishes, and were subsequently cleaned of fat and connective tissue and weighed to the nearest 0.01 mg. In some experiments dry weight was determined by drying the glands in an oven to constant weight (3 weighings).

ACTH was measured by radioimmunoassay (Dallman, DeManicor and Shinsako, 1974; Grizzle, *et al*., 1974; Rees, *et al*., 1974) and corticosterone was determined by competitive protein binding assay (Murphy, 1967) using human transcortin.

Statistically significance of the difference in means was calculated using Student's unpaired *t*-test.

C. RESULTS

Plasma ACTH and corticosterone levels, and the development of compensatory adrenal growth

Figure II-1 shows the results of this experiment. The operations were performed on groups of 6 rats under sodium methohexital anesthesia on two consecutive mornings. Groups of rats were killed at the indicated times after operations. Plasma ACTH levels in the left adrenalectomized rats are significantly elevated above the sham-operated controls only at 2 h ($p < 0.05$) and not before or after this time. Plasma corticosterone levels are significantly lower in left adrenalectomized than in sham-operated rats ¼, ½ and 1 h post-operatively ($p < 0.01$), and again at 8 h ($p < 0.05$). At 2, 4 and 12 h and at 1, 2, 3, and 10 d, plasma corticosterone levels are similar in the two groups. Right adrenal weight is significantly increased 12 h after left adrenalectomy ($p < 0.01$). The weight is increased but not significantly at 1 and 2 d ($p > 0.05$) followed by a large increase at 3 and 10 d ($p < 0.001$) compared to right adrenals of sham operated

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Fig. II-1. Circulating ACTH and corticosterone levels and right adrenal weight in male rats after left adrenalectomy or surgical sham operation under methohexital anesthesia. Each point represents the mean response of 6 rats. The vertical bars indicate ± SEM.

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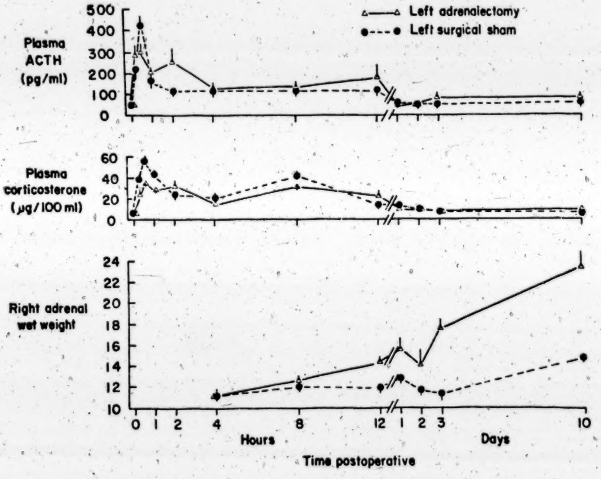
Left adrenalectomy
Left surgical sham
Plasma ACTH (pg/ml)
500
400
300
200
100
0
Plasma corticosterone (µg/100 ml)
60
40
20
0
Right adrenal wet weight
24
22
20
18
16
14
12
10
0
1
2
4
8
12
1
2
3
10
Hours
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rats.

To test whether the transient relative hypocorticoid signal, which is apparent during the first h after unilateral adrenalectomy under methohexital anesthesia, could be reduced or eliminated, we examined plasma corticosterone responses during the first 4 h after operations performed under sodium pentobarbital anesthesia. Pentobarbital anesthesia resulted in a considerable decrease in the magnitude and duration of the corticosterone response to sham adrenalectomy or unilateral adrenalectomy (Table II-1). However, there was still a relative hypocorticoid signal that persisted for 30 min in the adrenalectomy group; that is, the corticosterone response to the stress of surgery, while less than the response of sham adrenalectomized animals for 30 min, was elevated above resting levels at all times.

Inability of dexamethasone to prevent compensatory adrenal growth

To test whether the transient period of relative hypocorticoid response was a necessary signal to initiate compensatory adrenal growth, rats (6 per group) were treated with dexamethasone 2 h before operations were performed under ether anesthesia. Groups of rats were killed at 15 min, and 1, 2, 3 and 10 d later. At no time are plasma corticosterone levels above the normal resting range for male rats in the morning (Figure II-2). However, at 2 d, corticosterone levels in the surgical sham group are significantly higher than those in unilaterally adrenalectomized rats ($p < 0.05$). Nevertheless, adrenal weight is significantly increased by 1 d after unilateral adrenalectomy ($p < 0.01$), and the increase in weight that occurs during the 10 days of the study closely resembles the normal increase shown in Fig. II-1. These results not only showed that a relative hypocorticoid signal was

Table II-1. Plasma corticosterone response to left adrenalectomy or surgical sham adrenalectomy under pentobarbital anesthesia.

Time (h)	Plasma corticosterone (ug/100 ml)		P
0	5.7 ± 0.64(6)*		
	Adrenalectomy	Surgical Sham	
¼	14.4 ± 0.9 (6)	24.5 ± 2.5 (6)	0.005
½	18.8 ± 0.5 (6)	28.7 ± 1.1 (6)	0.001
1	18.1 ± 1.4 (6)	16.5 ± 1.8 (6)	ns
4	18.3 ± 2.0 (5)	13.7 ± 5.6 (4)	ns

*mean value ± 1 SEM (number of rats)

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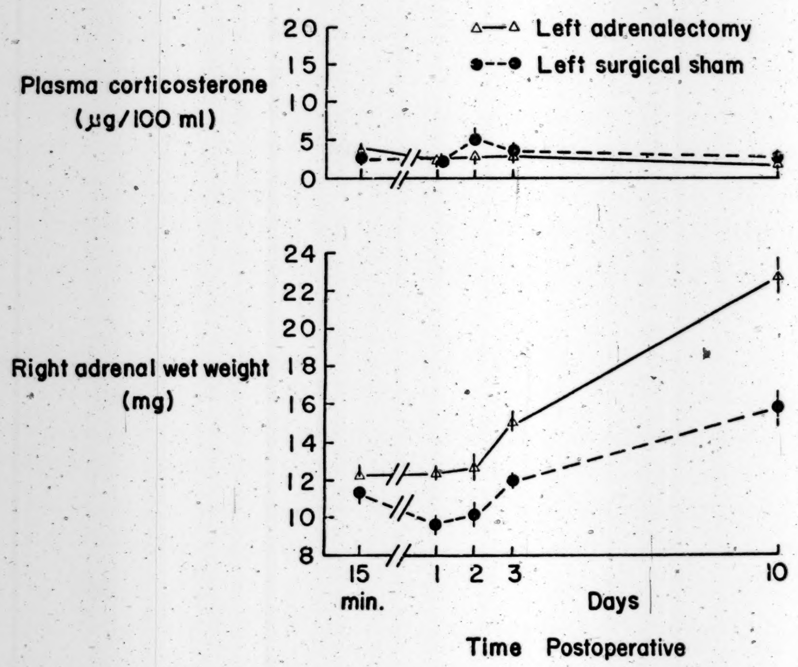
Fig. II-2. Circulating corticosterone levels and right adrenal weight after left adrenalectomy or surgical sham operation under ether anesthesia performed 2 h after rats were treated with dexamethasone (25 μg/100 g BW). Symbols and numbers of rats are as in Fig. II-1.


Left adrenalectomy
Left surgical sham
Plasma corticosterone (µg/100 ml)
20
15
10
5
0
Right adrenal wet weight (mg)
24
22
20
18
16
14
12
10
8
15 min.
1
2
3
10
Days
Time Postoperative

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unnecessary for compensatory adrenal growth to occur, but also suggested that increased ACTH secretion might not be necessary for the response. Dexamethasone treatment clearly inhibited the initial ACTH response to the operations as indicated by low plasma corticosterone levels at 15 min. The possibility remained, however, that ACTH secretion was occurring at an elevated rate at some period between 15 min and 1 day post-operatively to produce the significant increase in adrenal weight observed at that time.

Acceleration of compensatory adrenal growth by abolition of ACTH secretion with dexamethasone

To test the possibility that ACTH secretion was not required for compensatory adrenal growth, rats were pretreated with dexamethasone regimen known to inhibit the ACTH response to potent stimuli (Sirett and Gibbs, 1969). Groups of rats were treated with saline or dexamethasone 18 and 2 hours before and 22 hours after adrenalectomy or sham adrenalectomy. Other groups were similarly treated but not subjected to adrenal surgery. The latter groups were subjected to laparotomy with intestinal traction under ether anesthesia 10 min before killing to test the efficacy of the dexamethasone blockade of ACTH secretion. Plasma ACTH and corticosterone levels in dexamethasone treated rats are significantly lower ($p < 0.05$) than those in saline treated rats 24 h after left adrenalectomy or sham-operation (Figure II-3). In addition, by 6 h and at 24 h there is a highly significant increase in right adrenal weight in unilaterally adrenalectomized rats treated with dexamethasone ($p < 0.01$). The increase in right adrenal weight of the left adrenalectomized saline-treated group was not significant at 24 h compared to the sham-operated

Fig. II-3. Circulating ACTH and corticosterone levels and right adrenal weight 6 and 24 h after unilateral adrenalectomy or sham operation in rats treated with dexamethasone (25 μg/100 g BW) or saline (0.2 ml/100 g BW) at -18, -12, and +22 h. Bars represent the mean, horizontal lines above the bars indicate SEM, and the number of rats in each group is indicated at the bottom of each bar.

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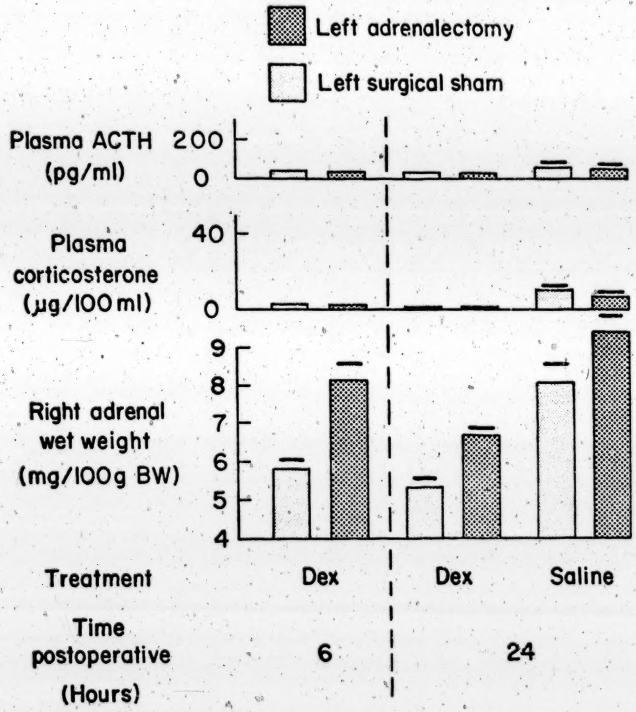
Left adrenalectomy
Left surgical sham
Plasma ACTH (pg/ml)
200
0
Plasma corticosterone (µg/100 ml)
40
0
Right adrenal wet weight (mg/100g BW)
9
8
7
6
5
4
Treatment
Dex
Dex
Saline
Time postoperative (Hours)
6
24

saline-treated control group ($p=0.08$). Significant compensatory adrenal growth by 6 h in dexamethasone treated rats was surprisingly rapid. Dry weights of right adrenals obtained 6 h after unilateral adrenalectomy or sham-operation also were significantly different ($p < 0.005$) (2.72 ± 0.11 and 1.98 ± 0.16 mg, respectively).

The normal rise in ACTH and corticosterone 10 min after laparotomy with intestinal traction is entirely inhibited in the dexamethasone treated rats (Table II-2). The dramatic adrenal atrophy produced after 42 h of dexamethasone treatment is apparent when the adrenal weights of saline and dexamethasone treated rats are compared (Figure II-3 and Table II-2).

Compensatory adrenal growth occurs in the absence of both a relative hypocorticoid signal and increased ACTH secretion. The results shown in Figure II-3 suggest that the growth may occur faster in the absence of ACTH secretion although by 24 h the adrenals of dexamethasone treated rats are smaller in absolute weight.

Compensatory adrenal growth following hypophysectomy

We next tested whether any hypophyseal secretions were required for compensatory adrenal growth by performing left adrenalectomy or sham adrenalectomy 2-4 h after hypophysectomy. Three days later rats (7 or 8 per group) were killed and the right adrenal glands were weighed. In both experiments right adrenals of left adrenalectomized rats were significantly heavier than right adrenals from sham adrenalectomized animals ($p < 0.01$, Expt. 1; $p < 0.02$, Expt. 2) (Figure II-4). Dry weights of adrenals from adrenalectomized and sham adrenalectomized rats in experiment 1 were 2.72 ± 0.153 and 1.892 ± 0.163 mg respectively, and the difference was highly significant ($p < 0.005$).

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Table II-2. Inhibition by dexamethasone of plasma ACTH and corticosterone responses 10 min after laparotomy with intestinal traction under ether anesthesia.

Time of dexamethasone (25 ug/100 g BW) or saline (0.2 ml/100 g BW) treatment before stress:		Plasma		Right Adrenal
		ACTH (pg/ml)	Corticosterone (ug/100 ml)	wet (mg/100 g BW) weight
-24 and -8 h	dexamethasone	34 ± 4*	4.4 ± 0.8	6.40 ± 0.37
	saline	508 ± 34	48.3 ± 3.6	6.93 ± 0.64
-42, -26 and -2 h	dexamethasone	26 ± 5	2.1 ± 0.1	4.87 ± 0.47
	saline	534 ± 43	47.0 ± 3.9	8.23 ± 0.25

*mean values for 6 rats are accompanied by ± 1 SEM

Fig. II-4. Compensatory adrenal growth 72 h after unilateral adrenalectomy in hypophysectomized or intact rats. The symbols used are as in Fig. II-3.

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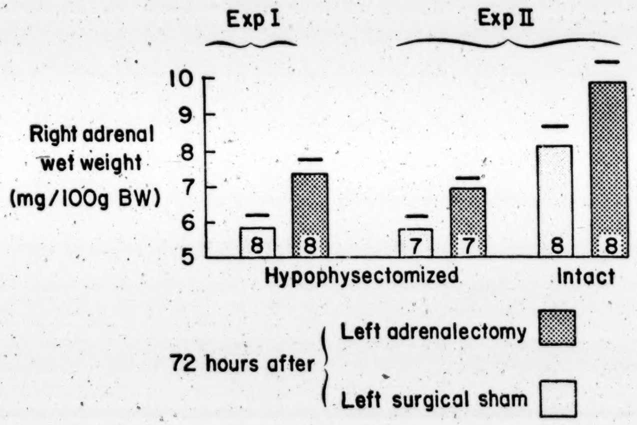
Exp I
Exp II
Right adrenal wet weight (mg/100g BW)
10
9
8
7
6
5
8
8
7
7
8
8
Hypophysectomized
Intact
72 hours after
Left adrenalectomy
Left surgical sham

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D. DISCUSSION

The results presented above show that neither a relative hypocorticoid signal nor increased ACTH (or hypophyseal) secretion is essential for compensatory adrenal growth to occur after unilateral adrenalectomy. This conclusion was suggested by finding only slight differences in plasma ACTH and corticosterone responses to unilateral adrenalectomy and sham adrenalectomy over the 10 d measurement period (Figure II-1). It was further supported by the finding that compensatory adrenal growth occurs normally in the dexamethasone treated rat in which the (ACTH and) corticosterone response(s) to operations were inhibited (Figure II-2).

Use of dexamethasone to inhibit entirely ACTH secretion during the 24 h period of observation resulted in atrophic adrenals at 24 h, as did hypophysectomy. Nonetheless, in both conditions compensatory adrenal growth (measured either by wet or dry weight) occurred after unilateral adrenalectomy when compared to similarly treated sham-operated controls. If adrenal weight of unilaterally adrenalectomized rats is expressed as a percentage of the adrenal weight of sham-operated controls, the degree of compensatory growth in the dexamethasone treated and hypophysectomized rats is similar to, or greater than that observed in saline-treated or unoperated animals at similar times after operations. These results may be interpreted as a decrease in the rate of atrophy caused by unilateral adrenalectomy after removal of ACTH. At 6 h, compensatory adrenal growth occurs prior to the onset of atrophy occasioned by removal of ACTH (shown by comparison of adrenal weights between dexamethasone-treated and saline-treated rats on Table II-2). We believe that the results of the 6 h dexamethasone experiment clearly show that ACTH is not required for compensatory adrenal growth, and that it is likely that we are observing the interaction between compensatory adrenal growth and removal of ACTH at later times.

Others also have found that unilateral adrenalectomy after hypophysectomy in the rat results in compensatory right adrenal growth when compared to right adrenals from sham-operated rats (Ganong, 1954; Lipscomb and Critchlow, 1966). However, when compared to the increase in adrenal weight produced by treatment with large doses of ACTH, the weight differences observed after unilateral adrenalectomy are slight (Ganong, 1954).

CHAPTER III

ACTH EFFECTS ON THE DEVELOPMENT OF COMPENSATORY ADRENAL GROWTH

A. INTRODUCTION

Investigation of compensatory adrenal growth after unilateral adrenalectomy has shown that neither a hypocorticoid signal nor the secretion of any pituitary hormone is required for the compensatory response. In addition, results from dexamethasone suppression experiments suggested that the abolition of ACTH secretion may accelerate the development of compensatory growth (Chapter II). It is surprising that ACTH, which is required to maintain adrenocortical weight in the hypophysectomized animal (Smith, 1930), could have a growth-inhibiting effect on the adrenal gland. Therefore, a series of experiments was performed to assess the effect on ACTH of adrenocortical growth after unilateral adrenalectomy; in addition to adrenal weight, these experiments determined the influence of ACTH on adrenal nucleic acid and protein content after unilateral adrenalectomy.

B. MATERIALS AND METHODS

Young male Sprague-Dawley rats (Simonsen, Gilroy, Ca) 100-150 g b.w. were received 1-4 d before use. The rats were housed 2-3/cage and in a temperature and light controlled room (lights on 0600-1800 h) and were allowed rat chow and water ad libitum. In some experiments rats were treated with dexamethasone (Decadron^R, Merck, Sharpe and Dohme) 25 ug/100 g b.w. subcutaneously at 18 and 2 h prior to adrenal surgery to prevent ACTH secretion in response to the operation. Hypophysectomy was performed by the parapharyngeal approach under ether anesthesia 2-4 h before adrenal surgery. In some experiments rats were injected with a long-acting ACTH preparation (Acthar gel, Armour) subcutaneously;

the regimen and amount of ACTH treatment varied and will be delineated below.

Rats were anesthetized with ether and the left adrenal gland was exposed through a retroperitoneal incision. Unilateral adrenalectomy consisted of removal of one adrenal whereas surgical sham adrenalectomy consisted of similar skin and muscle incisions and the adrenal gland was exposed but not touched. The skin incisions were clipped and rats were returned to their home cages. Rats were killed by decapitation at the times indicated below. Adrenals were removed and placed on ice in closed dishes on paper saturated with 0.9% NaCl. The chilled glands were cleaned of fat and connective tissue and weighed to the nearest 0.01 mg.

DNA and RNA were extracted from adrenal homogenates (1:100 w/v in distilled water) by the method of Schmidt and Thannhauser (1945), incorporating modifications recommended by Munro and Fleck (1966). Protein in 0.5 ml homogenate was precipitated by adding an equal volume of 10% trichloroacetic acid (TCA) followed by 2 washings of the precipitate with 5% TCA to remove acid soluble compounds. Lipids were removed by 2 washings with 95% ethanol saturated with NaAc followed by 2 washings with 95% ethanol saturated with NaAc:diethyl ether (3:1 v/v). The precipitate was dissolved in 0.5 ml 0.3N KOH for at least 2 h and then RNA was extracted by adding 0.5 ml cold 1N $HClO_4$. The supernatant was saved and combined with a further 0.5 ml wash with 0.5N $HClO_4$. RNA was assayed by the orcinol method in 0.5 ml samples. DNA was extracted by heating the precipitate at 70°C in 1.0 ml 0.5N $HClO_4$ for 20 min. A second wash of 0.5 ml was combined with the initial extract and 0.5 ml samples were measured in duplicate using the method of

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Burton (1968). Calf thymus DNA (Sigma) and D-ribose (Sigma) were used as standards for these determinations. Protein concentrations were determined by the method of Lowry (1951) using human albumin as standard.

In one experiment, adrenal growth was monitored by determining thymidine incorporation into adrenal DNA. Rats were anesthetized with ether and injected through a tail vein with ^{3}H-thymidine (New England Nuclear). Injections of labelled precursor (100 uC/100 g b.w. in 0.2 ml NaCl) were given at the time of operation or at 3 or 6 h following the operation. All animals were sacrificed 12 h following the operation; adrenals were removed, weighed, homogenized and nucleic acids were extracted as described above. Incorporation of label into total adrenal DNA was determined by counting 0.5 ml of the 0.5N $HClO_4$ DNA extract. At least 10,000 counts were collected; background activity was subtracted from each sample to compute cpm/total adrenal DNA.

Experiment I. Groups of 6 rats were treated with dexamethasone or saline before left adrenalectomy or sham adrenalectomy. All animals were sacrificed 6 h later. Right adrenal weight and DNA content were measured.

Experiment II. The design of this experiment is the same as that in Experiment I except that animals were sacrificed 24 h postoperatively and adrenal RNA and protein content were measured in addition to adrenal weight and DNA content.

Experiment III. Rats (8/group) were treated with dexamethasone before left adrenalectomy or sham adrenalectomy. An additional group of 7 rats was also given 8 U ACTH subcutaneously at the time of left adrenalectomy. All animals were sacrificed 12 h postoperatively. Adrenal weight, DNA and RNA content and thymidine incorporation into adrenal DNA were measured.

Experiment IV. Rats (6/group) were subjected to left adrenalectomy or left sham adrenalectomy and groups were killed 0, 1, 3 and 7 days later. Half of the rats received 4 U ACTH subcutaneously twice a day from the day of operation to the day of sacrifice; the remaining animals received saline injections at similar times. Adrenal weight, DNA and RNA content were determined.

Experiment V. Five groups of 16 rats were hypophysectomized 2-4 h before left adrenalectomy (8 rats/subgroup) or left sham adrenalectomy (8 rats/subgroup). An additional group with intact pituitaries was used as controls. All animals were sacrificed 3 days postoperatively. The intact control group and one hypophysectomized group received a saline injection at the time of operation whereas the 4 remaining hypophysectomized groups received 1.0 U ACTH subcutaneously at operation. In addition to the ACTH received at operation, 3 hypophysectomized groups received 0.5, 1.0 and 2.0 U ACTH, respectively, twice a day until sacrifice. Right adrenal wet weight was measured.

C. RESULTS AND DISCUSSION

I and II. Acceleration of the development of compensatory adrenal growth in the absence of ACTH. Table III-1 shows right adrenal weight and DNA content 6 h after left adrenalectomy. In animals treated with a regimen of dexamethasone shown to completely block ACTH secretion (Chapter II), right adrenal weight is elevated in left adrenalectomized rats compared to sham adrenalectomized controls at 6 h. This increase is paralleled by a significant increase in DNA content in adrenalectomized rats ($p < 0.01$ vs sham controls). In contrast, adrenalectomized animals not treated with dexamethasone failed to show an increase in

Table III-1. Right adrenal weight and DNA content 6 h after left adrenalectomy or left sham adrenalectomy. Dexamethasone treatment accelerates adrenal growth after unilateral adrenalectomy (n=6/group).

	right adrenal weight (mg)	DNA (ug/gland)
Dexamethasone-treated (25 ug/100 g b.w.) 18 and 2 h preoperatively		
sham adrenalectomy	8.49 ± 0.59	50.1 ± 4.8
left adrenalectomy	10.12 ± 0.72	78.0 ± 5.3*
Saline-treated (0.2 ml/100 g b.w.) 18 and 2 h preoperatively		
sham adrenalectomy	10.36 ± 0.82	63.9 ± 8.2
left adrenalectomy	9.32 ± 0.55	55.6 ± 4.5

*$p < 0.01$ vs dexamethasone-treated, sham adrenalectomy group and saline-treated, adrenalectomy group.

right adrenal weight or DNA content compared to sham adrenalectomized animals. No difference was found between adrenalectomized and sham adrenalectomized animals from either the dexamethasone-treated or saline-treated groups in adrenal RNA and protein content at 6 h (data not shown). These results complement earlier experiments which showed significant increases in adrenal wet and dry weight at 6 h after adrenalectomy (Chapter II); the rapid weight increase with concomitant increases in DNA content suggest that in the absence of ACTH adrenal hyperplasia is stimulated at 6 h after unilateral adrenalectomy.

Right adrenal weight, DNA, RNA and protein content 24 h after left adrenalectomy and sham adrenalectomy are shown in Figure III-1. By 24 h in dexamethasone-treated animals adrenal weight, DNA, RNA and protein content are significantly greater ($p < 0.05$) in adrenalectomized animals compared to sham adrenalectomized controls. The effectiveness of the ACTH inhibition by dexamethasone is apparent in comparing dexamethasone-treated and saline-treated groups. By 24 h in the absence of ACTH, adrenal weight is decreased; this decrease is paralleled by decreases in RNA and protein content. While the prominent influence of ACTH on adrenal growth is well demonstrated by the adrenal atrophy observed in its absence, compensatory growth is still observed when ACTH is removed.

In this experiment no difference in any adrenal parameter was found between the saline-treated adrenalectomized and sham adrenalectomized groups; however, in replicate experiments using animals not treated with dexamethasone, at 24 h after adrenalectomy significant increases were observed in some or all of these parameters (e.g. see Figure III-2). While an explanation for this variability is not

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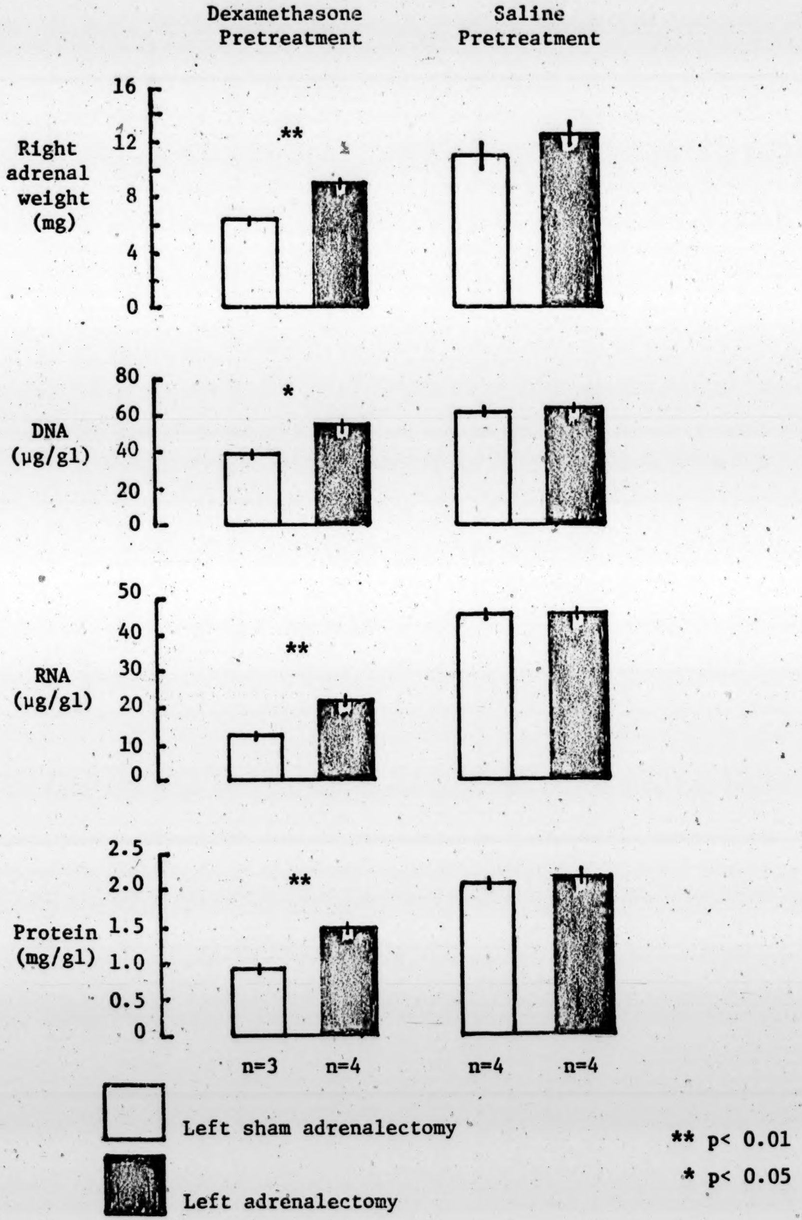
Fig. III-1. Right adrenal weight, DNA, RNA and protein content 24 h after left adrenalectomy or sham adrenalectomy in rats treated with dexamethasone (25 μg/100 g BW) or saline (0.2 ml/100 g BW). Bars represent the mean, vertical lines indicate ± SEM and the number of animals in each group is indicated at the bottom of each column.


Dexamethasone Pretreatment
Saline Pretreatment
Right adrenal weight (mg)
16
12
8
4
0
**
DNA (µg/gl)
80
60
40
20
0
*
RNA (µg/gl)
50
40
30
20
10
0
**
Protein (mg/gl)
2.5
2.0
1.5
1.0
0.5
0
**
n=3
n=4
n=4
n=4
Left sham adrenalectomy
Left adrenalectomy
** p< 0.01
* p< 0.05

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apparent, the amount of ACTH secreted at the time of operation may be important in dictating how quickly compensatory growth will be expressed. An increased initial exposure to circulating ACTH may suppress subsequent adrenal growth after unilateral adrenalectomy.

III. Decreases in adrenal weight, DNA content and ^{3}H-thymidine incorporation following replacement of dexamethasone-treated animals with ACTH. Since dexamethasone treatment suppresses the secretion of other pituitary hormones in addition to ACTH under certain experimental conditions (Reichlin, 1974; Florsheim, 1974), it was possible that the removal of hormones other than ACTH by dexamethasone was responsible for accelerating compensatory growth. To test this hypothesis, the effect of ACTH replacement of dexamethasone-treated animals was studied. Table III-2 shows right adrenal weight, DNA and RNA content and thymidine incorporation into adrenal DNA 12 h after left adrenalectomy, sham adrenalectomy and left adrenalectomy with ACTH replacement. Whereas right adrenal weight, DNA and RNA content and thymidine incorporation were significantly elevated ($p < 0.05$) in left adrenalectomized animals compared to sham adrenalectomized animals, treatment with ACTH eliminated or suppressed these differences. Right adrenal weight and thymidine incorporation following ACTH treatment were not different between adrenalectomized and sham adrenalectomized groups, and DNA content was significantly suppressed ($p < 0.05$) in ACTH-replaced, adrenalectomized rats compared to non-replaced, adrenalectomized animals. Since increased DNA content and thymidine incorporation into DNA indicate the occurrence of cell proliferation, these results suggest that the growth response after unilateral adrenalectomy is primarily hyperplastic and that circulating ACTH can suppress this type of

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Table III-2. Right adrenal weight, DNA and RNA content and ^{3}H-thymidine incorporation 12 h after left adrenalectomy and left sham adrenalectomy in dexamethasone-treated animals. All animals were treated with dexamethasone (25 ug/100 g b.w.) 18 and 2 h preoperatively. ACTH replacement decreases adrenal weight, DNA content and thymidine incorporation.

	Right adrenal weight (mg/100 g b.w.)	^{3}H-thymidine incorporation (cpm/DNA)	DNA (ug/100 g b.w.)	RNA (ug/100 g b.w.)
sham adrenalectomy (n=8)	7.54 ± 0.39	222 ± 35	49.0 ± 2.6	38.7 ± 2.2
left adrenalectomy (n=7)	9.58 ± 0.84*	344 ± 40*	71.7 ± 2.4**	54.0 ± 2.6*
left adrenalectomy with ACTH replacement (8 U sc at operation) (n=8)	8.48 ± 0.44	274 ± 34	63.0 ± 2.5*	59.1 ± 2.7*

*significantly greater than sham adrenalectomy controls $p < 0.05$.

**significantly greater than adrenalectomy plus ACTH replacement $p < 0.05$.

adrenal growth.

IV. Delay of the development of compensatory adrenal growth after ACTH treatment. Since a single injection of ACTH at the time of operation can suppress compensatory growth at 12 h, experiments were performed to test whether prolonged ACTH treatment could delay the development of compensatory adrenal growth. Figure III-2 shows right adrenal weight, DNA and RNA content after left adrenalectomy and left surgical control operation in animals treated with saline or 4 U ACTH twice daily for the duration of the experiment. In the saline-treated groups, adrenal weight, DNA and RNA content are significantly increased in adrenalectomized animals at 1, 3 and 7 days postoperatively ($p < 0.01$ or $p < 0.05$ vs surgical controls). However, ACTH treatment completely inhibits weight increases in adrenalectomized animals ($p > 0.5$ vs surgical controls) until 7 days postoperatively; this inhibition was paralleled by no differences in DNA content throughout the 7 day period. RNA content was elevated in the ACTH-treated, adrenalectomized animals at 1 and 7 days ($p < 0.05$ and $p < 0.01$, respectively). Comparison of saline-treated, and ACTH-treated, surgical control groups shows that adrenal weight is increased by ACTH treatment at 1, 3 and 7 days; this increase can probably be attributed to increased RNA content at these same times. These data support the observations of many laboratories that ACTH treatment causes rapid and large increases in adrenal RNA and protein content (Farese and Reddy, 1963; Bransome and Chargaff, 1964; Imrie et al., 1965) leading to the conclusion that ACTH stimulates adrenal weight increases through cell hypertrophy, not hyperplasia. The increased DNA content observed after 7 days of ACTH treatment in this experiment was not unexpected in light of similar findings by

Fig. III-2. Right adrenal weight, DNA and RNA content at the time of left adrenalectomy or sham adrenalectomy and at 1,3 and 7 days after the adrenal operations in rats treated with saline (0.2 ml/100 g BW) or ACTH (4 U) twice daily. Solid circles and open triangles represent the mean response of 6 rats and the vertical lines indicate ± SEM.

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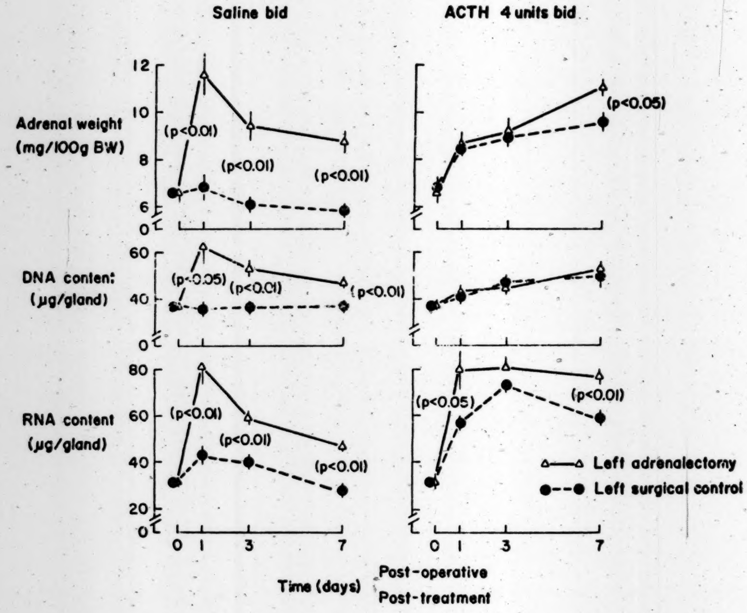
Saline bid
ACTH 4 units bid
Adrenal weight (mg/100g BW)
12
10
8
6
0
(p<0.01)
(p<0.01)
(p<0.01)
(p<0.05)
DNA content (µg/gland)
60
40
0
(p<0.05)
(p<0.01)
(p<0.01)
RNA content (µg/gland)
80
60
40
20
0
(p<0.01)
(p<0.01)
(p<0.01)
(p<0.05)
(p<0.01)
0
1
3
7
Time (days)
Post-operative
Post-treatment
Left adrenalectomy
Left surgical control

other workers (Bransome and Reddy, 1961; Fiala, Sproul and Fiala, 1956). These investigators explained the cell proliferation after prolonged ACTH treatment according to the theory of "critical mass"; that is, mitosis in a cell is inevitable when the cytoplasmic subcellular elements attain a critical mass. Thus, cell proliferation stimulated by ACTH secretion in these experiments probably contributes little to the initiation and development of compensatory growth.

V. Inhibition of compensatory adrenal growth in hypophysectomized animals by ACTH treatment. Experiments were performed to determine how much ACTH was required to suppress the development of compensatory adrenal growth. Figure III-3 shows right adrenal weight in intact and hypophysectomized animals 3 days after left adrenalectomy and sham left adrenalectomy. Intact and hypophysectomized animals receiving saline at the time of adrenal surgery showed normal compensatory growth at 3 days. Loss of pituitary hormones decreased the absolute adrenal weights of hypophysectomized animals compared to intact animals; however, significant differences ($p < 0.05$) in adrenal weight were still found between adrenalectomized and sham adrenalectomized animals within both groups. The remaining hypophysectomized groups which received different amounts of ACTH did not show compensatory growth. Surprisingly, a single injection of 1 U ACTH at the time of gland removal had as great an inhibitory effect as any of the other ACTH regimens. These findings indicate that increased circulating ACTH at the time of adrenal surgery is sufficient to delay compensatory adrenal growth for 3 days.

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Fig. III-3. Right adrenal weight 72 h after left adrenalectomy or sham adrenalectomy in intact or hypophysectomized rats treated with saline (0.2 ml/100 g BW) at the time of adrenal surgery. Additional groups of hypophysectomized rats were treated with ACTH (1.0 U) at the time of adrenal surgery in addition to saline or 0.5, 1.0 or 2.0 U ACTH twice daily. Bars represent the response of 8 rats and the vertical lines indicate ± SEM.

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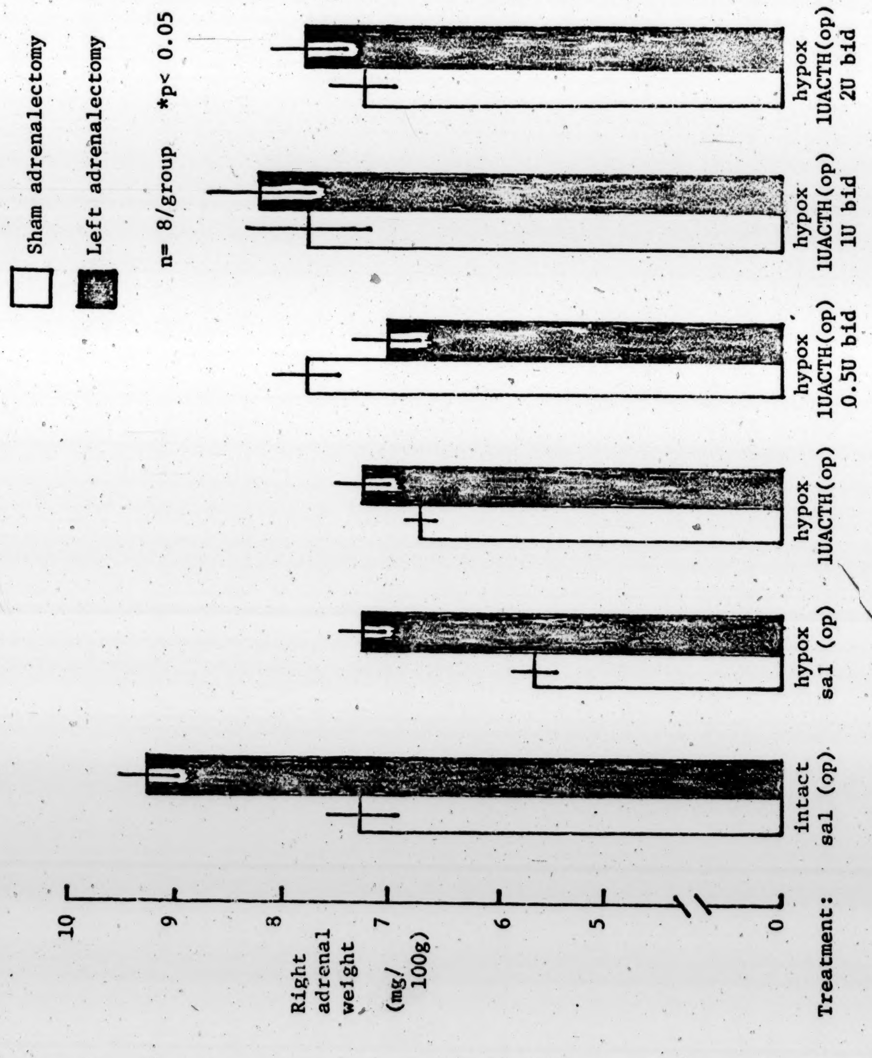
Sham adrenalectomy
Left adrenalectomy
n= 8/group *p< 0.05
Right adrenal weight (mg/100g)
10
9
8
7
6
5
0
Treatment:
intact sal (op)
hypox sal (op)
hypox 1UACTH(op)
hypox 1UACTH(op) 0.5U bid
hypox 1UACTH(op) 1U bid
hypox 1UACTH(op) 2U bid

D. CONCLUSION

These experiments confirm earlier work (Bransome and Reddy, 1961; Reiter and Pizzarello, 1966) showing that unilateral adrenalectomy results in cell proliferation in the remaining adrenal gland. Adrenal DNA content is increased by 24 h in the presence of ACTH (Figure III-2) and by 6 h in the absence of ACTH (Table III-1). It is consistent with these results to classify adrenal weight increases after unilateral adrenalectomy as "compensatory adrenal hyperplasia" rather than "compensatory adrenal hypertrophy". While significant increases in RNA content occur after unilateral adrenalectomy (Figure III-2), these changes can probably be ascribed to the action of ACTH stimulated by the stress of surgery and not to the stimulus initiated by adrenalectomy.

The body of data presented in this chapter extends earlier experimental results which showed that ACTH secretion is not required for compensatory adrenal growth (Chapter II). While ACTH treatment increases adrenal weight and RNA and protein content, it suppresses the hyperplastic response to unilateral adrenalectomy. Although the mechanism by which ACTH inhibits cell growth is unknown, the large and rapid increase in RNA and protein content could play a role. Activation of DNA synthesis has been attributed to the synthesis of a template RNA which codes the enzymes involved in the separation of the DNA strands and the polymerization of the component deoxynucleotides (Baserga, 1965). In addition, inhibition of RNA synthesis a few hours before initiation of DNA synthesis inhibits subsequent DNA synthesis (Schwartz, Sternberg and Phillips, 1963). ACTH stimulation, by initiating the synthesis of RNA used as a template to assemble enzymes

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required for adrenal steroidogenesis (Gill, 1972), may decrease the pool of RNA precursors within the cell; that is, the cell may not have the materials necessary to initiate cell proliferation in response to unilateral adrenalectomy. Thus, the increased RNA content after ACTH treatment may reflect the mechanism responsible for delaying compensatory growth.

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CHAPTER IV

NEURAL MEDIATION OF COMPENSATORY ADRENAL GROWTH

A. INTRODUCTION

The finding that dexamethasone pretreatment or hypophysectomy did not prevent compensatory adrenal growth after unilateral adrenalectomy in rats suggested that neither a hypocorticoid signal nor hypersecretion of ACTH is required for the compensatory response. The failure of hypophysectomy to eliminate the response to unilateral adrenalectomy further suggested that a non-humoral factor might be responsible for compensatory growth. Since neural activity has been implicated in some tissues to explain the capacity for specific compensatory growth following tissue loss (Muir, Pollock and Turner, 1975; Ashrif, Gillespie and Pollock, 1974), studies were designed to determine whether nerves mediate the compensatory adrenal response after unilateral adrenalectomy. A series of experiments was performed in which suspected afferent and/or efferent adrenal neural pathways were interrupted, and the response to subsequent unilateral adrenalectomy was measured. Lesioned areas included: hypothalamic nuclei which were reported to receive afferent neural activity from the adrenal (Sajonski et al., 1965; Halasz and Szentagothai, 1959; Gerendai _et_ _al_., 1974); the spinal cord at a thoracic level (T-2) above the entrance or exit of splanchnic nerve fibers; the splanchnic nerve as it enters or leaves the celiac ganglion; and the vagus nerve at cervical level. Since all lesions would undoubtably affect tissues in addition to the adrenal gland, an effect on the adrenal resulting from a lesion might be secondary to alterations produced on another tissue. Thus, unilateral lesions were made in all experimental series; a unilateral adrenal response produced by a unilateral lesion is probably due to altering adrenal neural activity directly whereas a bilateral effect can be considered to constitute a secondary effect of the lesion.

B. MATERIALS AND METHODS

Male Sprague-Dawley rats (Simonsen, Gilroy, CA) weighing 100-150 g were used in these studies. Animals, received 1-5 days before experiments, were housed 2 per cage in an artificially lighted room with an automatically controlled light cycle (lights on from 06.00 h until 18.00 h). Food and water were available at all times. Surgery was performed under ether or 5 mg/100 g b.w. sodium pentobarbital (Nembutal^R, Abbott) anesthesia. Unilateral adrenalectomy was performed through a dorsal midline skin incision and a lateral retroperitoneal incision. Sham unilateral adrenalectomy was performed through similar incisions and the gland was observed but not touched.

Hypothalamic lesions. Unilateral hypothalamic lesions were produced by passing anodal DC current through a unipolar platinum electrode insulated with epoxylite, except at the tip (diameter 0.25 mm). All lesions were produced by passing a current of 1 mA for 30 sec. The electrode was placed into the hypothalamus of the sham-lesioned rats but no current was passed. Rats were lesioned on the left or right side of the hypothalamus, or they were sham-lesioned unilaterally; 4 days later either the left or right adrenal gland was removed. An additional group of rats was unilaterally sham-adrenalectomized. All rats were killed 4 days after unilateral adrenalectomy or unilateral sham adrenalectomy. At the time of sacrifice brains were removed and fixed in 10% formalin. 50μ sections of frozen brains were stained with carbol fuchsin and subsequently studied microscopically to determine the site of the lesions.

Spinal cord hemi-sections. Hemi-section of the spinal cord was performed through a dorsal midline skin incision and separation of muscle layers overlying the vertebral column. Following removal of connective tissue and dura matter surrounding the spinal cord, the cord was punctured in the midline of the intervertebral space between the 2nd and 3rd thoracic vertebrae using a 27-gauge hypodermic needle. Using the needle perforation as a placement guide, one blade of a micro-scissors was placed through the cord and half the cord was sectioned horizontally; left or right hemi-sections were made. At sacrifice spinal cords were removed and fixed in 10% formalin. 15-μ paraffin sections were cut and stained. Every tenth section from each cord was stained for myelin using the Kluver-Barrera technique; the remaining sections were stained with carbol fuchsin. Sections were subsequently studied microscopically to determine the extent of the lesion. Sham hemi-section consisted of similar skin and muscle incisions and removal of connective tissue layers and dura mater but the spinal cord was not touched. Unilateral adrenalectomy or unilateral sham adrenalectomy was performed 5-7 days following hemi-section; all rats were sacrificed 3 days following the adrenalectomy surgery.

Adrenal denervations. For all denervation surgery, the adrenal gland and surrounding vascular and neural elements were exposed through lateral skin and lateral retroperitoneal incisions. Figure IV-1 shows a diagram of the left adrenal gland and surrounding neural elements as viewed in the rat from a dorsolateral approach. All exposures and denervations were performed with the aid of a Bausch and Lomb dissecting

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Fig. IV-1. Diagram of the rat adrenal gland illustrating the adrenal nerve supply and major blood vessels.

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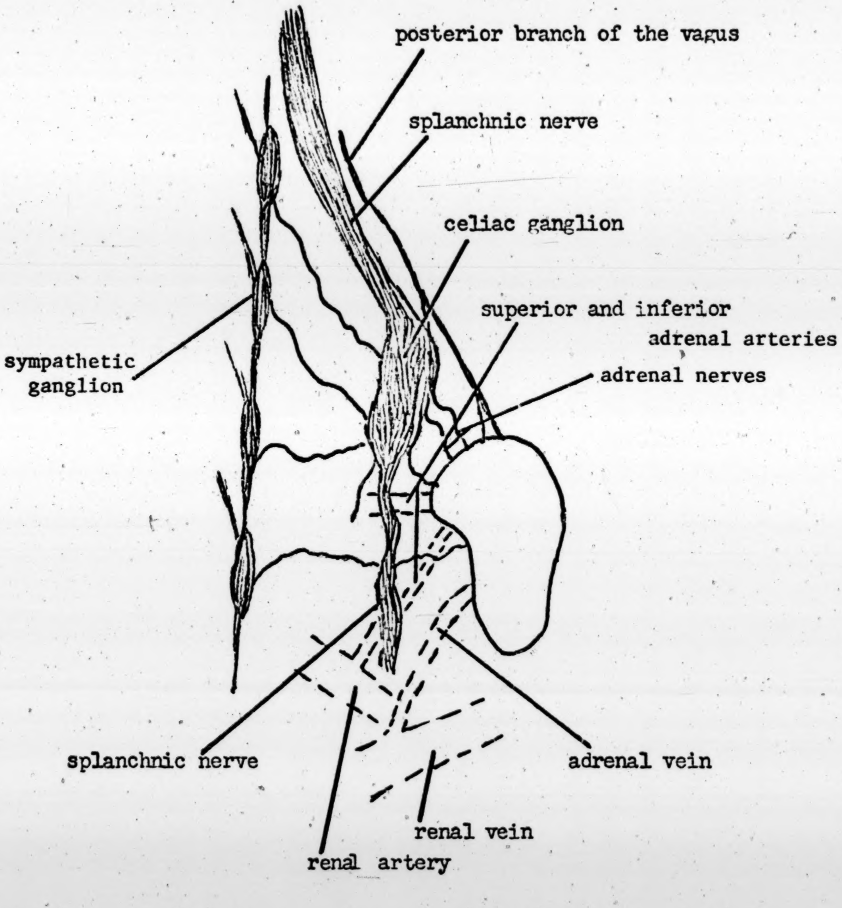
posterior branch of the vagus
splanchnic nerve
celiac ganglion
superior and inferior
adrenal arteries
adrenal nerves
sympathetic
ganglion
splanchnic nerve
adrenal vein
renal vein
renal artery

microscope. The sham denervation consisted of retracting the left adrenal gland from its position at the anterior pole of the kidney and exposing the left celiac ganglion, the splanchnic nerves and adrenal nerves which lie medial to the adrenal gland. Celiac isolation consisted of retracting the left adrenal gland, exposing the celiac ganglion and cutting the splanchnic nerve both rostral and caudal to the celiac ganglion. This procedure removes splanchnic input from the celiac ganglion; input from vagal fibers, adrenal fibers and fibers from the sympathetic chain may not be affected by this cut. Splanchnectomy consisted of manipulations similar to celiac isolation; however, only splanchnic fibers travelling rostral to the celiac ganglion were severed. Adrenal nerve transection consisted of exposing and cutting any fibers observed between the celiac ganglion and the adrenal. The small size of these fibers, however, limited the probability of successfully interrupting this pathway. For all denervation experiments, rats were unilaterally adrenalectomized or unilaterally sham adrenalectomized 5 days following the operations. All rats were sacrificed 3 days after the adrenalectomy or sham adrenalectomy surgery.

Cervical vagotomy. Following a ventral midline skin incision, muscle layers overlying the carotid sheath were separated at the level of the cricoid cartilage. The vagus nerve was exposed unilaterally, retracted from its position between the internal jugular vein and common carotid artery, and severed. Sham unilateral vagotomy consisted of a similar exposure of the vagus without touching the nerve. Animals were unilaterally adrenalectomized or unilaterally sham adrenalectomized 2-3 days after the surgery; all rats were sacrificed 3 days following

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the adrenalectomy operation.

Lidocaine blockade. Under ether anesthesia, the left adrenal gland was exposed through a dorsal midline skin incision and lateral retroperitoneal incision. The adrenal gland was retracted laterally and lidocaine (Xylocaine, 1% lidocaine HCl, Astra) or saline was placed on the adrenal blood vessels and nerves by injecting 0.2 ml into the exposed area and by placing a cotton swab saturated with lidocaine or saline adjacent to the adrenal pedicle. Five min following drug or vehicle administration, the cotton swab was removed and the left adrenal was removed or returned to its initial position. Skin incisions were closed with wound clips and animals were returned to home cages. All animals were killed 12 hours later.

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In all lesion experiments, animals were killed by decapitation and the adrenals were immediately removed and collected on filter paper saturated with 0.9% NaCl in closed dishes; subsequently, adrenals were cleaned of fat and connective tissue and weighed to the nearest 0.01 mg. In some experiments, adrenals were placed in an oven at $100^{o}C$ and dried to constant weight.

Statistical significance of the difference in means was calculated using Student's _t_-test.

C. RESULTS AND DISCUSSION

Blockade of compensatory adrenal growth by unilateral hypothalamic lesions

Table IV-1 shows the results of the hypothalamic lesion experiments. Individual rats are listed in the table and are grouped according to whether adrenalectomy was on the same side as or the side opposite to the lesion. The side of adrenalectomy is indicated, and the animals are assigned to one of four subgroups according to the site of the lesion. There is no significant difference between the mean adrenal weight of all rats with lesions on the side of the hypothalamus contralateral to the side of adrenalectomy and the mean adrenal weight of sham-lesioned unilaterally adrenalectomized rats ($p > 0.5$); compensatory adrenal growth occurs normally in this group. In contrast the remaining adrenal gland from rats with lesions ipsilateral to the side of gland extirpation weighs significantly less than those either from sham-lesioned unilaterally adrenalectomized controls ($p < 0.025$) or from contralaterally-lesioned unilaterally adrenalectomized rats ($p < 0.005$).

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Table IV-1 Comparison of the effects and sites of lesions placed ipsilaterally and contralaterally to the side of adrenalectomy

Controls:	Mean adrenal wt. (mg/100 g b.w.)
No lesion; sham-unilateral adrenalectomy	7.00 ± 0.61 (6)
Sham lesion; unilateral adrenalectomy	9.58 ± 0.46 (6)

Coordinates [de Groot, 1959]	Ipsilateral lesions			Contralateral lesions		
	Animal No.	Side of adrenalectomy	Adrenal wt., mg/100 g b.w.	Animal No.	Side of adrenalectomy	Adrenal wt., mg/100 g b.w.
A 5.4						
vertical:	15	L	8.6	5	L	9.2
−3.0 to −3.6	19	L	5.7	26	R	11.1
lateral:	21	R	7.9	37	R	13.2
0.7 to 1.3	36	R	6.7	38	R	10.6
Mean ± SEM			7.23 ± 0.64			11.03 ± 0.83
			(t = 3.624) p = 0.011			
A 5.4	8	L	8.3	3	L	9.5
vertical:	10	L	8.3	9	L	9.8
−3.6 to −4.7	14	L	6.7	11	L	11.7
lateral:	22	R	7.2	18	L	10.7
0.7 to 1.3	23	R	7.7	24	R	7.1
	30	R	9.7	25	R	9.5
	39	R	8.9	29	R	7.8
Mean ± SEM			8.11 ± 0.38			9.44 ± 0.60
			(t = 1.860) p = 0.086			
A 4.6						
vertical:	12	L	8.9	13	L	9.2
−3.0 to −3.7	21	R	7.9	37	R	13.2
lateral:	27	R	10.4	38	R	10.6
0.7 to 1.6						
Mean ± SEM			9.07 ± 0.73			11.00 ± 1.17
			(t = 1.402) p = 0.233			
A 4.6	2	L	7.5	7	L	10.1
vertical:	4	L	5.6	24	R	7.1
−3.7 to −4.4	6	L	5.3	26	R	11.1
lateral:	8	L	8.3	37	R	13.2
0.7 to 1.6	10	L	8.3	38	R	10.6
	17	L	5.6			
Mean ± SEM			6.77 ± 0.58			10.42 ± 0.98
			(t = 3.335) p = 0.009			

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Coordinates according to the atlas of de Groot (1959) were arbitrarily chosen to divide lesioned areas into dorsal and ventral areas. The total extent of lesions falling within each brain area is shown schematically in Figures IV-2 and IV-3 for levels A 5.4 and 4.6, respectively; individual adrenal weights are given in Table IV-1. At the more anterior level it is clear that dorsal lesions ipsilateral to adrenalectomy inhibit compensatory adrenal growth compared to dorsal lesions contralateral to adrenalectomy (p=0.011). Ventrally placed lesions at this anterior level do not appear to affect compensatory adrenal growth. Figure IV-2 shows the total vertical and lateral extent of the lesions at A 5.4. Figure IV-4 shows two examples of these lesions at their greatest extent: on the top is a lesion that was followed by ipsilateral adrenalectomy and no compensatory adrenal growth; on the bottom is a lesion that was followed by contralateral adrenalectomy and normal compensatory adrenal growth.

More posteriorly, lesions placed dorsally do not inhibit compensatory adrenal growth whether they are placed ipsilaterally or contralaterally to the side of subsequent adrenalectomy. There is no significant difference between either group and the control, sham-lesioned unilaterally adrenalectomized rats (p=0.55 and p=0.21 for ipsilaterally- and contralaterally-lesioned animals vs controls, respectively). In contrast, ipsilateral lesions placed ventrally result in a significantly decreased adrenal weight compared to the adrenal weight of the sham-lesioned unilaterally adrenalectomized rats (p=0.009). Figure IV-3 shows the total vertical and lateral extent of lesions made at this hypothalamic level. Figure IV-5 shows two examples of lesions at their greatest extent: as in Figure IV-4

Fig. IV-2. Total vertical and lateral extent of lesions placed in the ventral hypothalamus at anterior-posterior level A 5.4 (de Groot). Dashed lines denote specific hypothalamic nuclei and shaded areas correspond to the first two groups of coordinates in Table IV-1.

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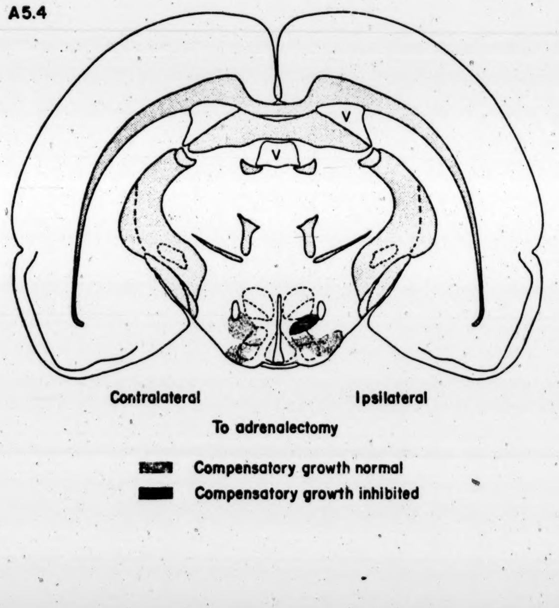
A5.4
V
V
Contralateral
Ipsilateral
To adrenalectomy
▨ Compensatory growth normal
■ Compensatory growth inhibited

Fig. IV-3. Total vertical and lateral extent of lesions placed in the ventral hypothalamus at anterior-posterior level A 4.6 (de Groot). Symbols are as in Fig. IV-2.

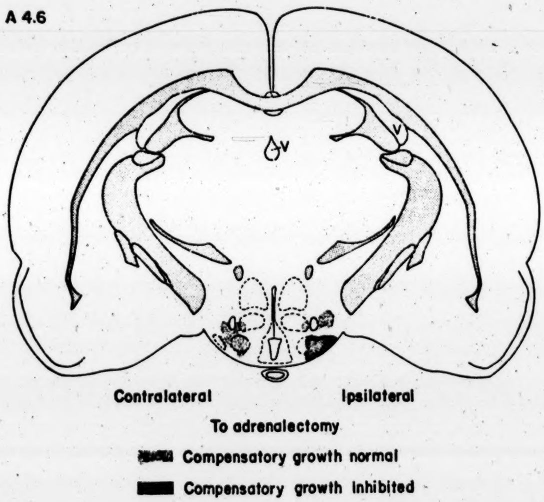
A 4.6
V
V
Contralateral
Ipsilateral
To adrenalectomy
▨ Compensatory growth normal
■ Compensatory growth inhibited

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Fig. IV-4. Photomicrographs of hypothalamic lesions at level A 5.4 in 2 rats (100 x magnification). The micrograph on the top shows a lesion made ipsilaterally to subsequent adrenalectomy that prevented compensatory adrenal growth. The micrograph on the bottom shows a lesion at the same level and of approximately the same size that was contralateral to the side of subsequent adrenalectomy. Compensatory adrenal growth was normal in this rat.

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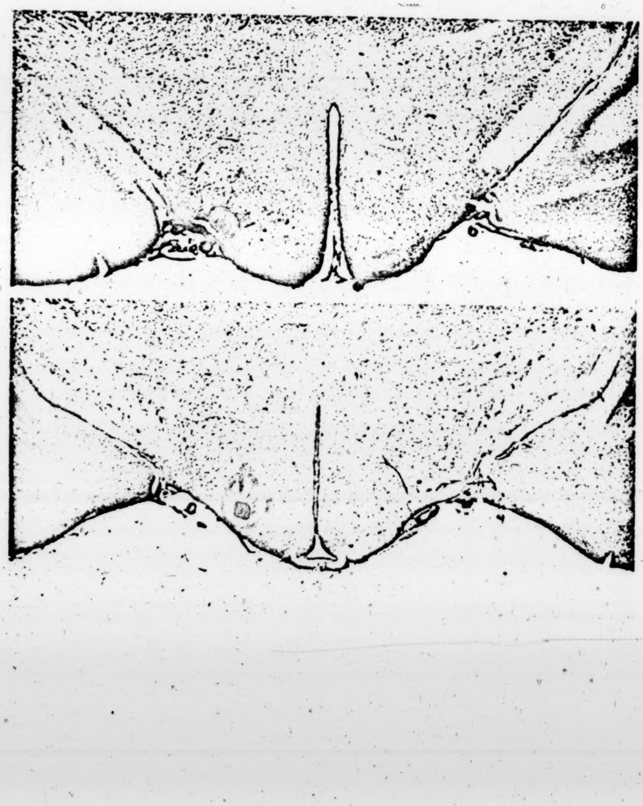

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Fig. IV-5. Photomicrographs (100 x magnification) of hypothalamic lesions at level A 4.5. As in Fig. IV-4, the top micrograph shows a lesion made on the side ipsilateral to the removed adrenal and on the bottom is shown a micrograph of a lesion on the side contralateral to the removed adrenal. Compensatory adrenal growth was prevented by the lesion made ipsilaterally to the removed adrenal but not by the contralateral lesion.

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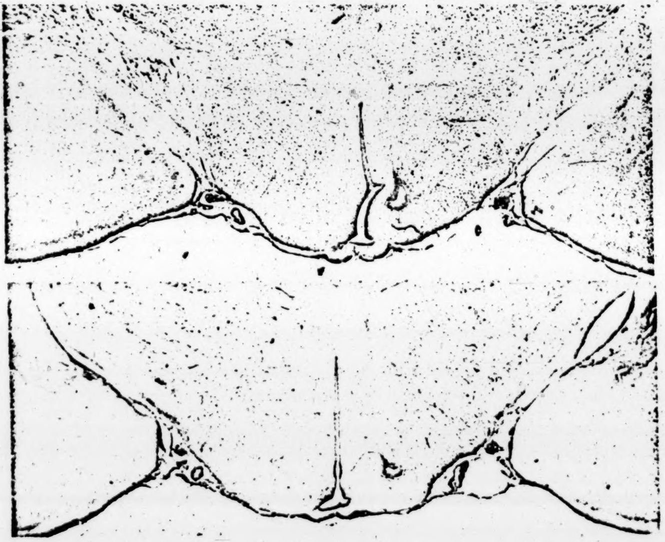

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one lesion (top) is on the side ipsilateral to the removed adrenal gland and inhibits compensatory adrenal growth; the other lesion is on the side contralateral to the removed adrenal gland and does not interfere with compensatory adrenal growth.

Compensatory adrenal growth in these experiments was measured by determining the wet weight of the remaining adrenal four days after unilateral adrenalectomy. It was shown previously that, compared to sham operated controls, there is an increase of approximately 25% in adrenal weight 3 days after unilateral adrenalectomy. This percentage difference in weight was sustained but not markedly increased 10 days post-operatively (Chapter II). The 4-day endpoint in these experiments was chosen for convenience.

Because of the crossed design of the present experiments, both left and right adrenals were used to determine whether compensatory adrenal growth had occurred. In this strain of rats we have not been able to measure a significant difference in weight between the left and right adrenal glands, although the left tends to be heavier by about 4%. When alternate adrenals were collected from 56 unstressed male rats weighing 120-140 g, the left and right adrenals weighed 10.91 ± 0.32 (N=28) and 10.44 ± 0.35 (N=27) mg, respectively. The difference in weight was not significant. Similarly, in a series of pairs of adrenals collected 12 h after sham adrenalectomy, left and right adrenals weighed 9.49 ± 0.51 and 9.17 ± 0.44 mg, respectively (N=16); the difference was not significant using a paired *t*-test (t=1.40). Therefore the increased weight of either adrenal above the weight observed in sham-operated controls probably reflects compensatory growth, and conversely, no increase in weight above the level in

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sham-operated controls probably denotes the lack of compensatory growth.

The results of these experiments strongly suggest that compensatory adrenal growth is neurally mediated by a crossed efferent pathway originating in or passing through the hypothalamus, to ultimately terminate in the contralateral adrenal gland. Because the study was performed using a crossed design, it is clear that only a lesion that is ipsilateral to the removed adrenal will prevent compensatory adrenal growth. Lesions placed ipsilaterally to the side of adrenalectomy in the area of the dorsal lateral part of the ventromedial nucleus anteriorly and the lateral premammillary nucleus posteriorly completely prevent the compensatory response. Ipsilateral lesions adjacent to these areas, in addition to contralateral lesions throughout the ventral hypothalamus, do not affect compensatory adrenal growth. The sites of the effective lesions are compatible with the course of the medial forebrain bundle.

Bilateral hypothalamic lesions that produce bilateral adrenal atrophy (Endroczi and Mess, 1955; Szentagothai, _et al_., 1962) or prevent compensatory adrenal growth (Bachrach and Kordon, 1958) have been reported. Although these adrenocortical changes were ascribed to decreases in ACTH secretion, the atrophy may have resulted from disruption of efferent nerves to the gland. This interpretation is supported by the finding that the location of such lesions is similar, although greater in extent, to the location of the lesions in this study. Furthermore, electrical simulation of this hypothalamic region does not cause acute ACTH secretion, as measured by depletion of adrenal ascorbic acid (Endroczi, Kovacs and Lissak, 1955). In addition, unilateral lesions in the same hypothalamic area lead to a decrease in the nuclear volume of zona fasciculata cells on the side contralateral to the lesion (Smollich and Docke, 1969). In sum, these results support the hypothesis that there is an efferent neural pathway from the hypothalamus to the contralateral adrenal gland that regulates adrenal cortical growth.

Blockade of compensatory adrenal growth by hemi-section of the spinal cord at thoracic level-2.

If a neural pathway from the hypothalamus to the adrenal gland mediates adrenal growth after unilateral adrenalectomy, destruction of efferent pathways from the hypothalamus should prevent compensatory growth. To test this hypothesis, the compensatory growth response was examined after spinal cord hemi-section lesions. The results of the hemi-section experiments are shown in Figure IV-6. For each hemi-section group, the number of animals that underwent right adrenalectomy or right sham operation equalled the number that underwent left adrenalectomy or left sham operation. Ipsilateral hemi-section refers to animals whose spinal cords were hemi-sected on the same side as the adrenalectomy or sham adrenalectomy,whereas contralateral hemi-section refers to animals which were hemisected on the side opposite to subsequent adrenal surgery. The spinal cord surgery was performed 5-7 days prior to the adrenal surgery. In sham hemi-sected animals there was a significant increase ($p < 0.01$) in adrenal weight after adrenalectomy when compared to the adrenal weight of unilaterally sham adrenalectomized animals. Likewise, following an adrenalectomy ipsilateral to the side of cord hemi-section, compensatory growth did occur. However, the response to unilateral adrenalectomy was blocked in animals which were adrenalectomized on the side contralateral to

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Fig. IV-6. Right adrenal weight 72 h after unilateral adrenalectomy or sham adrenalectomy in rats in which spinal cords had been hemi-sected at thoracic level-2 or sham hemi-sected. Bars represent the mean, vertical lines indicate ± SEM and the number of animals in each group is indicated at the base of each bar.

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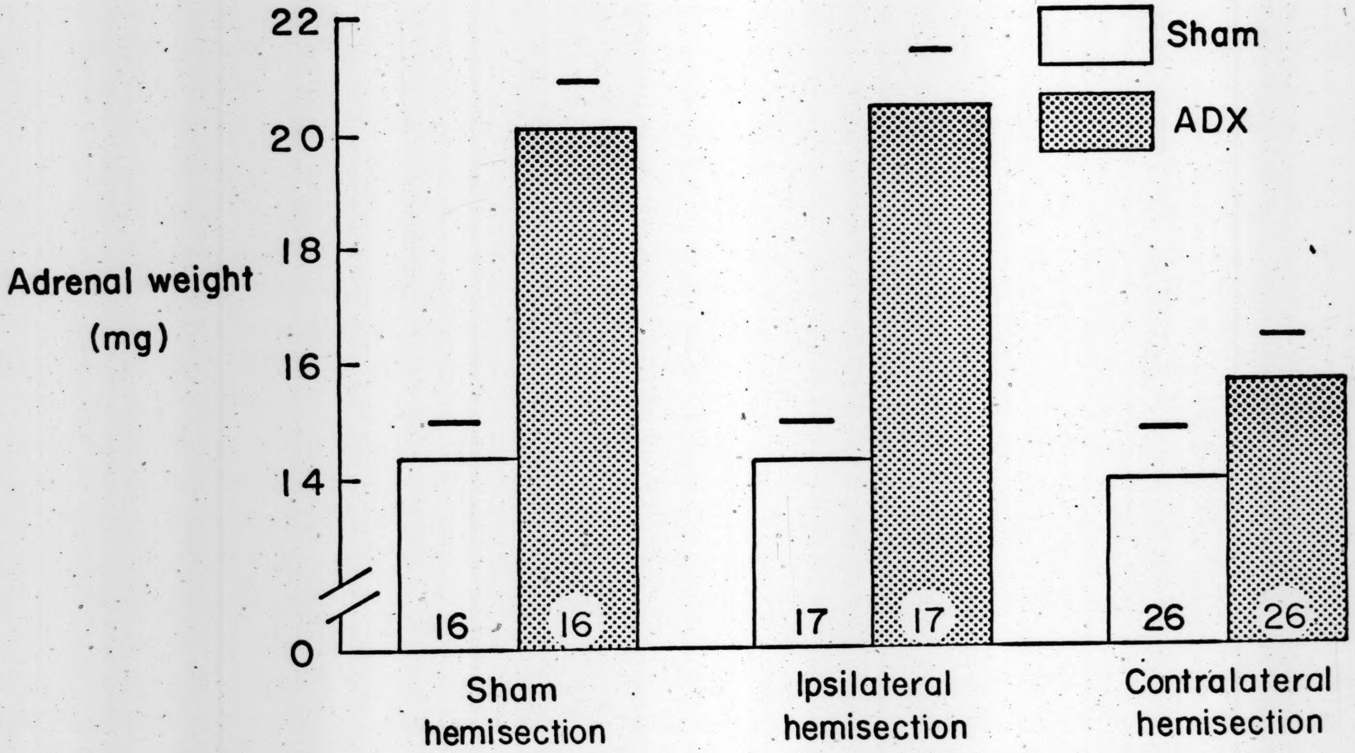
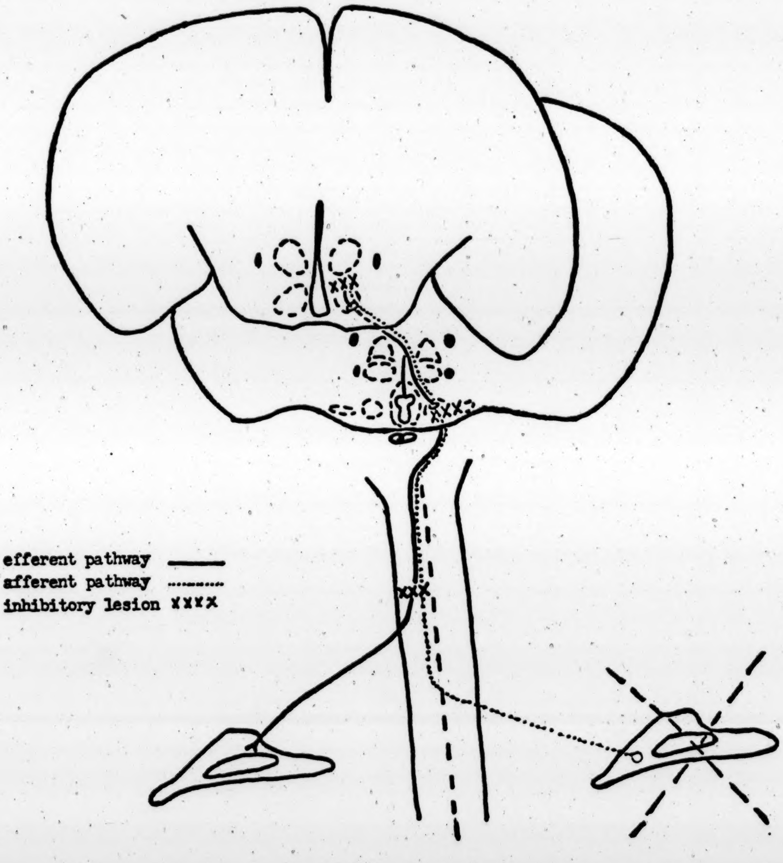

Sham
ADX
Adrenal weight
(mg)
22
20
18
16
14
0
16
16
17
17
26
26
Sham
hemisection
Ipsilateral
hemisection
Contralateral
hemisection

the cord hemi-section. There was no significant difference ($p > 0.50$) in adrenal weight between contralateral-lesioned, unilaterally adrenalectomized animals and contralateral-lesioned, unilaterally sham adrenalectomized controls. In addition, the response to unilateral adrenalectomy was significantly suppressed in the contralateral-lesioned group compared to the ipsilateral- hemi-sected group ($p < 0.01$) and the sham hemi-sected group ($p < 0.01$). Histological data from animals having partial hemi-sections or from animals with lesions extending across the midline were not included in the statistical analyses. The finding that hemi-section of the spinal cord on the contralateral side inhibits compensatory adrenal growth whereas hemi-section on the ipsilateral side has no inhibitory effect suggests that the spinal cord lesion is interrupting a strictly neural pathway; that is, the sidedness of the effective lesion does not support the possibility that secretion of a humoral agent is being affected by the operation.

The sketch in Figure IV-7 summarizes the hypothalamic lesion and spinal cord hemi-section data. Lesions in the dorsal part of the ventromedial nucleus anteriorly or in the ventral and lateral premammilary nuclei posteriorly block compensatory adrenal growth if placed in the hypothalamus ipsilateral to the side of adrenalectomy; lesions of the spinal cord block compensatory adrenal growth if the hemi-section is contralateral to the side of adrenalectomy. Together these results reveal that compensatory adrenal growth is neurally mediated; that the compensatory response is regulated centrally; and that either an afferent component, or an efferent component, or both afferent and efferent components are neurally mediated.

If the assumption is made that removing the adrenal gland

Fig. IV-7. Diagram of the proposed neural pathway mediating compensatory adrenal growth after unilateral adrenalectomy. The solid line represents the efferent pathway, the dotted line represents the afferent pathway and the X's indicate the position of lesions which blocked compensatory growth.


efferent pathway
afferent pathway
inhibitory lesion XXXX

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stimulates growth of the contralateral gland by severing afferent neural connections, cutting the hypothetical afferent nerves should result in adrenal growth. Using the increase in weight of the initially removed adrenal as a criterion to determine afferent neural stimulation, no hypothalamic lesion or spinal cord lesion produced in these experiments stimulated afferent adrenal nerves. Thus, lesions which inhibit compensatory growth are cutting efferent pathways. Alternatively, the lesions may be cutting both efferent and crossed afferent components; in this case, severing the efferent component removes any possibility of identifying the crossed afferent component. The conclusion from these studies as represented in the figure is that the efferent component of the compensatory response is neural having its origin in the brain, crossing the midline, descending in the spinal cord and terminating in the adrenal gland. While there is little evidence for an afferent neural component, consideration of a humoral agent as the afferent component also remains speculative.

Failure of peripheral nerve transection to alter the compensatory growth response

The hypothalamic lesion and spinal cord hemi-section data suggested that adrenal nerves were necessary for compensatory adrenal growth. Attempts were made to follow the efferent pathway from the spinal cord to the adrenal by cutting nerves known to send fibers to the adrenal gland. Also, if the afferent components travelled with the efferent component within the central nervous system, the two pathways might separate in the periphery and become susceptible to identification through selective lesions. Unfortunately, the complexity of the adrenal innervation and the close proximity of

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neural elements to the adrenal blood supply in the rat (see Figure IV-1) made the denervation surgery difficult. Denervations were performed which insured that the adrenal vasculature was not compromised. Based on this criterion, at no time were we convinced that all adrenal neural elements had been severed by any single intervention. Studies from other laboratories (Hata, 1959; Aburaya and Hata, 1961) have reported results based on adrenal denervation data which suggest that denervated adrenals were atrophied and hypofunctioning compared to innervated adrenals. However, there is no convincing evidence that the results obtained were due solely to cutting adrenal nerves rather than to compromising the adrenal blood supply. Future studies performed in larger animals may circumvent this problem.

Results of denervation experiments performed on the rat are shown in Table IV-2. Compensatory adrenal growth was studied by comparing the left adrenal weight of right adrenalectomized animals to the left adrenal weight of right sham adrenalectomized animals. In both control groups and in all experimental groups, compensatory adrenal growth after unilateral adrenalectomy occurred normally; by both wet and dry weight determinations, adrenal weight was elevated in adrenalectomized animals compared to sham adrenalectomized controls.

While compensatory growth could not be inhibited by any surgical intervention at the level of the adrenal gland, the denervation experiments led to some unexpected findings. For example, the celiac isolation procedure on the left side decreased adrenal weight on the left side in right adrenalectomized animals and on both sides in right sham adrenalectomized animals when compared to corresponding adrenals from sham denervation groups, splanchectomy groups and adrenal nerve

Table IV-2. Comparison of the effects of peripheral nervous system lesions on the adrenal weight of right adrenalectomized and right sham adrenalectomized animals.

	Adrenal Wet (Dry) Weight (mg ± SE) Post Right Adrenalectomy		Adrenal Wet (Dry) Weight (mg ± SE) Post Right Sham Adrenalectomy		
	N	left	N	left	right
Control groups:					
No surgery	6	16.8 ± 0.9** (4.2 ± 0.2)**	6	12.9 ± 1.3 (3.2 ± 0.3)	12.9 ± 1.4 (3.2 ± 0.4)
Sham denervation	21	17.6 ± 0.9 ** (4.4 ± 0.3)**	19	13.4 ± 0.4 (3.3 ± 0.1)	13.0 ± 0.4 (3.3 ± 0.1)
Experimental groups:					
Celiac isolation	11	14.6 ± 0.8*** (3.7 ± 0.2)***	12	11.5 ± 0.6* (2.8 ± 0.1)*	11.1 ± 0.5* (2.8 ± 0.1)*
Splanchnectomy	8	22.3 ± 1.4*** (5.8 ± 0.4)***	8	14.0 ± 0.6 (3.2 ± 0.2)	14.2 ± 0.8 (3.3 ± 0.2)
Adrenal nerve cut	15	17.5 ± 0.9** (4.4 ± 0.2)**	7	12.9 ± 0.7 (3.2 ± 0.2)	12.9 ± 0.7 (3.3 ± 0.2)

* $p < 0.05$ vs corresponding adrenal weight from sham denervation group.

** $p < 0.05$ vs left adrenal weight from corresponding right sham adrenalectomy groups.

cut groups. The decreased adrenal weight of the celiac isolation group was also apparent in examining the right adrenal removed at the time of right adrenalectomy; the initially removed adrenal weighed significantly less than the initially removed adrenal of the other groups ($p < 0.05$). On the other hand, the splanchnectomized group of animals had significantly greater adrenal weights after unilateral adrenalectomy compared to the sham denervation group ($p < 0.05$). The manifestation of this increased left adrenal weight was dependent on removal of the right adrenal, since the initially removed right adrenal did not differ in weight from similar adrenals from the other groups, nor were the adrenal weights of the splanchnectomy groups significantly different from the other groups after sham adrenalectomy. These data do not support a pre-adrenalectomy effect of splanchnectomy.

This is in contrast to the celiac isolation experiments in which adrenal weight was decreased compared to controls prior to adrenalectomy. These findings are interpreted as suggesting that cutting the splanchnic nerve rostral to the celiac ganglion removes an inhibitory input to the adrenal and that this input manifests itself only in response to unilateral adrenalectomy. Since splanchnic fibers are known to innervate adrenal blood vessels (Hollinshead, 1936), cutting the splanchnic nerve could alter adrenal blood flow. If vasomotor effects prevented a blood-borne agent normally inhibitory to compensatory growth from affecting adrenocortical cells, compensatory growth could be potentiated. Studies from this laboratory suggest that ACTH can inhibit increased DNA synthesis stimulated by unilateral adrenalectomy (Chapter III). Thus, decreased exposure to ACTH or other growth inhibiting substances might explain the significantly increased

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compensatory growth in splanchnectomized animals. However, at this time there are few data to support this speculation and until the proper experiments are completed, the splanchnectomy effect cannot be explained.

Cutting rostrally and caudally to the celiac ganglion results in decreases in adrenal weight. The bilateral change in adrenal weight suggests that alteration of a humoral signal is involved. Since the splanchnic nerve bundle caudal to the celiac ganglion distributes fibers to many visceral structures, including the gut and pancreas, hormone secretion from these structures which could be altered by caudal splanchnectomy might account for the changes in adrenal weight. Measuring changes in insulin and glucagon secretion after splanchnectomy might test the merit of this hypothesis. Since this has not been done, questions related to celiac isolation effects on adrenal weight remain unanswered.

Results of the adrenal nerve transection experiments showed that the attempt to sever nerve fibers between the celiac ganglion and the adrenal gland was completely unsuccessful; adrenal weight in these animals was no different from sham denervation groups or unoperated controls.

In conclusion, results from the denervation experiments do not clearly support earlier findings which suggested that compensatory adrenal growth is neurally mediated. While some interesting but as yet unexplainable effects were produced by cutting nerves known to send fibers to the adrenal, adrenocortical growth after unilateral adrenalectomy was not altered. A possible explanation for this finding is that the adrenal gland was not sufficiently denervated following any of the surgical interventions. Early work by Young (1939) showed

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that complete denervation of the adrenal medulla in rats was accomplished only following numerous lesions which included severing the splanchnic nerve and removing the sympathetic chain from the diaphragm down to the ganglion which gives rise to the transverse ramus at lumbar-4. Swinyard (1937) and Young (1939) showed that cutting the splanchnic nerve rostral to the celiac ganglion denervated only 1/3 to 1/2 of the adrenal medulla. In addition, since a large percentage of visceral nerves travel with the vasculature of the innervated tissue (Hollinshead, 1956), denervation without cutting blood vessels would leave visceral nerves intact. Thus, denervation attempts described in this chapter would remove only a portion of the adrenal innervation. Support for the contention that extensive adrenal denervation is a necessity is provided by the spinal cord hemi-section results. Cutting the spinal cord at a thoracic level above the outflow to the adrenal was designed to interrupt the majority of connections between the brain and the adrenal gland; this procedure was effective in blocking the growth response after unilateral adrenalectomy.

Inability of vagotomy to alter the compensatory response after unilateral adrenalectomy

Kirshenblat and Chigrina (1969) reported evidence that parasympathetic neural activity could alter adrenal weight in rats. Following bilateral vagotomy, adrenal weight increased bilaterally. Since vagotomy alters the function of numerous systems, including cardiovascular, respiratory and gastrointestinal, the parasympathetic effect on adrenal weight might be secondary to vagal influences on one of these systems. However, vagal fibers have been followed to the celiac ganglion (McCrea, 1924) and directly to the adrenal gland

(Pick, 1970). Therefore, a series of unilateral vagotomy experiments was performed to examine the possibility that direct adrenal innervation via the vagus might affect adrenal growth. In the initial vagotomy experiment the response to unilateral adrenalectomy was compared in sham vagotomized, left vagotomized and right vagotomized animals (Figure IV-8). In all three groups, the increase in left adrenal weight after right adrenalectomy was greater than the weight increase after sham right adrenalectomy; that is, compensatory adrenal growth occurred normally in all groups. Surprisingly, however, the adrenal weight of right vagotomized animals was decreased compared to the corresponding adrenal weights of the other two groups. This unexpected result prompted us to replicate the initial experiment. As shown in the middle panel, the effect of right vagotomy could not be repeated; in addition, compensatory growth responses in all groups were diminished and were not significantly elevated compared to sham adrenalectomy responses.

This latter finding was subsequently explained when we found that the animal room light cycle had been altered during this experiment and that the animals had lived under conditions of constant light. While this occurrence seemed tragic at the time, the serendipitous finding that light cues might alter the magnitude of the compensatory response may prove to be important in unraveling the mechanism(s) responsible for compensatory growth. The pineal gland, whose secretions are influenced by the light-dark cycle in rats (Wurtman and Cardinali, 1974), synthesizes a molecule called vasotocin which has been shown to inhibit compensatory growth after unilateral adrenalectomy (Pavel, Matresch and Petresch, 1973). Future work may reveal some connection

Fig. IV-8. Left and right adrenal weight 72 h after unilateral adrenalectomy or sham adrenalectomy in 3 separate experiments in which rats had been unilaterally vagotomized or sham vagotomized 3 days prior to adrenal surgery. Bars represent the mean response of 6 animals, vertical lines indicate ± SEM and the side of the weighed adrenal is noted below each bar.

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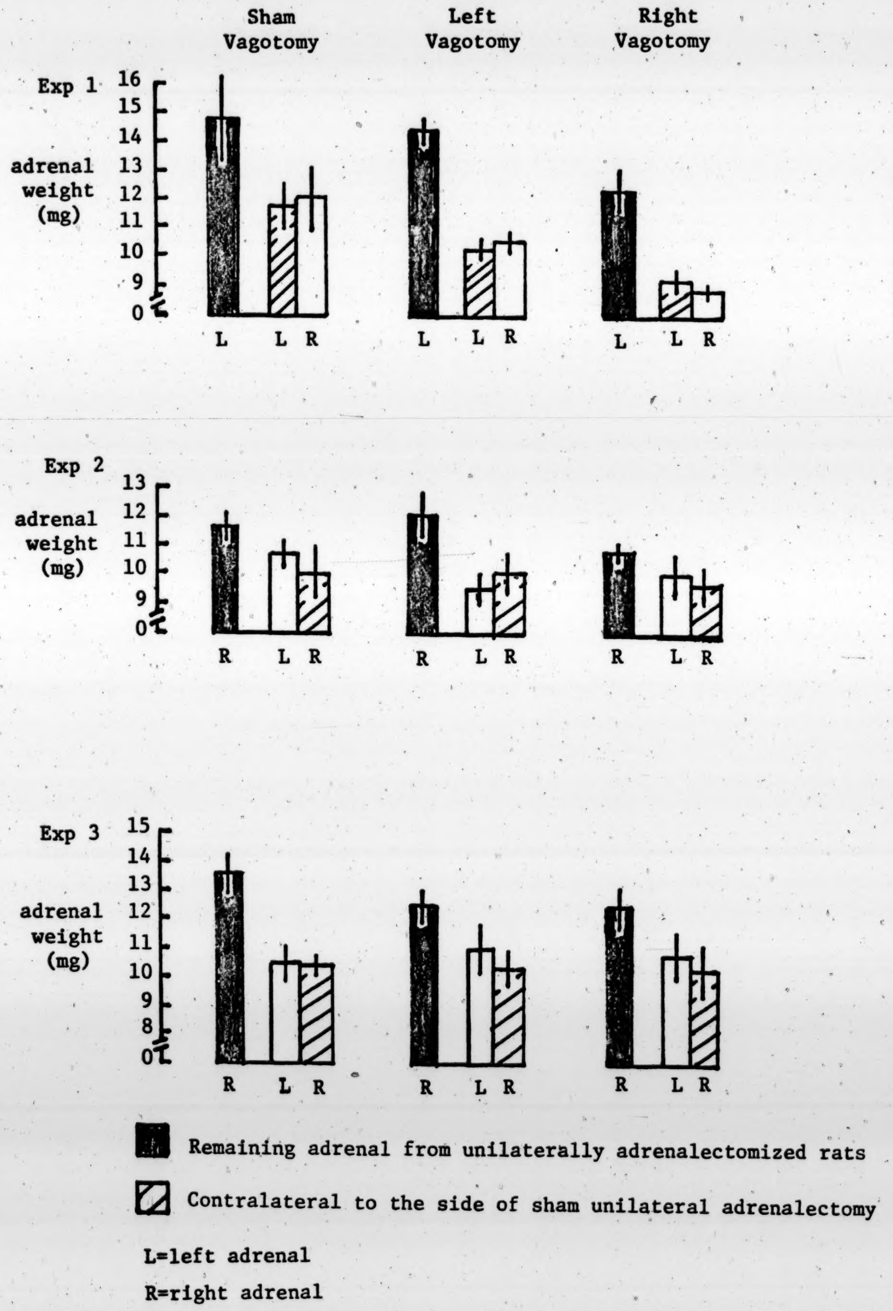
Sham Vagotomy
Left Vagotomy
Right Vagotomy
Exp 1
adrenal weight (mg)
16
15
14
13
12
11
10
9
0
L
L R
L
L R
L
L R
Exp 2
adrenal weight (mg)
13
12
11
10
9
0
R
L R
R
L R
R
L R
Exp 3
adrenal weight (mg)
15
14
13
12
11
10
9
8
0
R
L R
R
L R
R
L R
Remaining adrenal from unilaterally adrenalectomized rats
Contralateral to the side of sham unilateral adrenalectomy
L=left adrenal
R=right adrenal

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between light cues, pineal function and adrenocortical growth.

Since the second vagotomy experiment had been performed under altered lighting conditions, another attempt was made to repeat the findings of the initial vagotomy experiment. As shown in the bottom panel of Figure IV-8, compensatory growth occurred normally in all groups and there was no significant difference in adrenal weight among any of the groups. While right vagotomy lowered adrenal weight in one experiment, this effect could not be replicated in subsequent experiments.

Unilateral vagotomy had no consistent effect on adrenal weight in these experiments. The failure of these studies to show an increase in adrenal weight similar to the increases reported by earlier workers (Kirshenblat and Chagrina, 1969) suggests that the bilateral effects on adrenal weight were not the result of removing direct adrenal innervation but probably secondary to alteration of other vagally innervated structures. The finding that unilateral vagotomy did not affect adrenal weight unilaterally supports the hypothesis that vagal input to the adrenal does not play a physiological role in the maintenance of adrenal weight.

Blockade of the afferent neural component and inhibition of compensatory adrenal growth by lidocaine treatment

The finding that unilateral hypothalamic lesions and spinal cord hemi-sections could block compensatory adrenal growth supported the hypothesis that adrenal growth after unilateral adrenalectomy is neurally mediated. Based on the assumption that cutting afferent nerves should stimulate adrenal growth and not inhibit it, the hypothalamic and spinal cord effects were defined as lesions interrupting

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the efferent component of the compensatory response. In addition, cutting peripheral nerves did not provide evidence to support a role for afferent adrenal nerves in the growth response. Since the lack of success in affecting compensatory growth by cutting peripheral nerves might be explained by the inability to completely denervate the adrenal gland, an experiment was performed using a short-acting, local anesthetic to test whether a more complete block of adrenal nerves could alter the compensatory response. Results of this experiment showed that control animals treated with saline had a normal compensatory response 12 hours after unilateral adrenalectomy ($p< 0.05$ vs sham unilaterally adrenalectomized controls)(figure IV-9). However, whereas treatment with lidocaine had no effect on the right adrenal weight of sham adrenalectomized animals ($p > 0.05$ vs saline-treated, sham adrenalectomized animals), lidocaine treatment completely blocked the 12 hour adrenal response of unilaterally adrenalectomized animals ($p > 0.05$ vs lidocaine treated, sham adrenalectomized animals; $p < 0.05$ vs saline-treated, left adrenalectomized animals). The blockade of compensatory growth by pretreatment of the removed adrenal with a neural blocking agent clearly demonstrates the neural nature of the afferent component. In addition, the finding that treatment of adrenal nerves with a blocking agent on one side that does not stimulate adrenal growth on the contralateral side suggests that removal of neural activity (presumably, removal of inhibitory activity) is not the mechanism by which removal of one adrenal stimulates growth of the remaining adrenal. Instead, adrenalectomy by cutting adrenal nerves increases neural activity at the contralateral adrenal by providing excitatory input. Taken together, the results of the hypothalamic

Fig. IV-9. Left and right adrenal weight 12 h after left adrenalectomy or sham adrenalectomy in rats in which the left adrenal had been treated with saline or Xylocaine. Bars represent the mean response, vertical lines indicate ± SEM and the number of animals in each group is indicated at the base of each bar.

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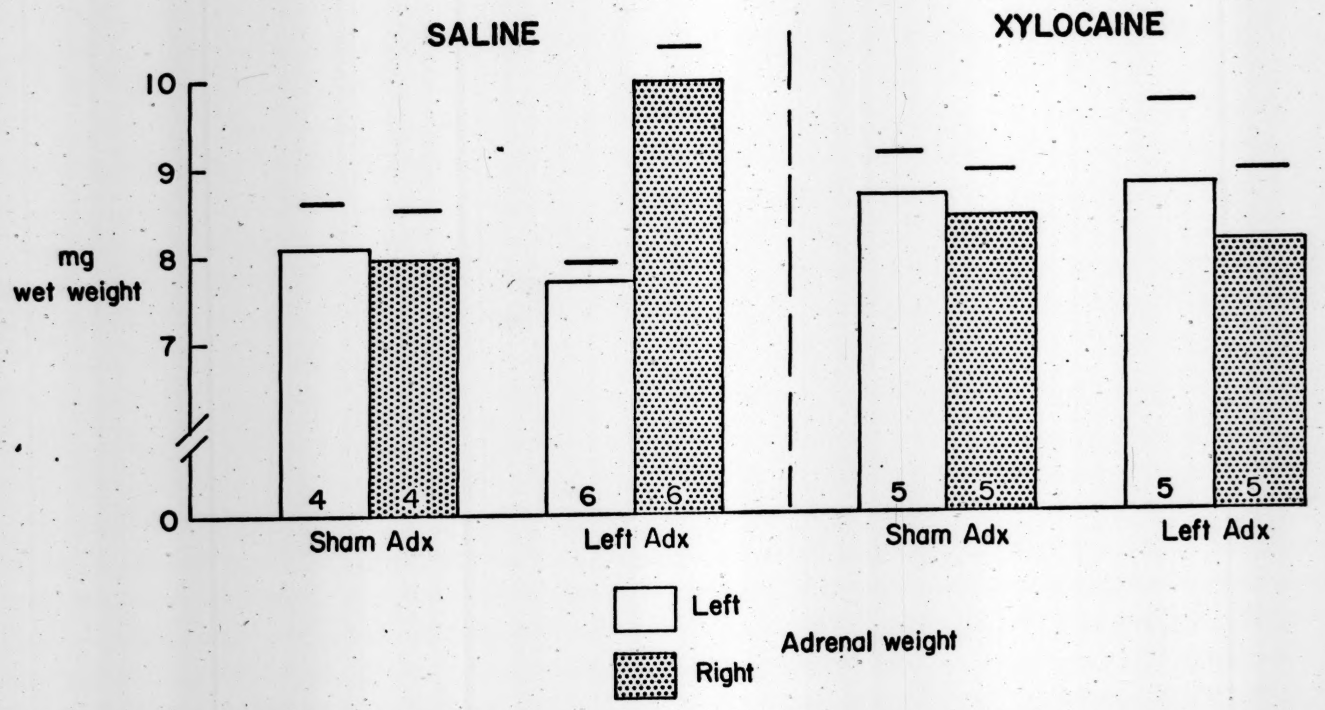
SALINE
XYLOCAINE
10
9
8
7
0
mg
wet weight
4
4
6
6
5
5
5
5
Sham Adx
Left Adx
Sham Adx
Left Adx
Left
Right
Adrenal weight

lesion experiments, the spinal cord hemi-section experiments and the lidocaine blockade experiments demonstrate the existence of a neural pathway from one adrenal gland to the hypothalamus which crosses and descends in the spinal cord to the other adrenal; the adrenal growth observed after unilateral adrenalectomy is mediated by increases in neural activity arising within this pathway.

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CHAPTER V

COMPENSATORY ADRENAL GROWTH AS A NEURALLY MEDIATED REFLEX

A. INTRODUCTION

The hypothesis that adrenal growth after unilateral adrenalectomy is neurally mediated is supported by experiments in which neural connections between the brain and the adrenal gland were severed and adrenal growth was inhibited. However, these findings have not elucidated the nature of the afferent component in compensatory adrenal growth. While evidence cited earlier (Niijima and Winter, 1968a, b; Halasz and Szentagothai, 1959; Kiss, 1951) confirms the existence of afferent adrenal nerves, no physiological role has been found for these elements. Recently, additional evidence for afferent adrenal nerves has been found in our laboratory (Sato et al., 1975). In that study, which examined changes in hypothalamic CRF content with time after application of ACTH-releasing stimuli, changes in CRF content occurred within seconds of completion of bilateral adrenalectomy that seemed unique to the stimulus of touching the adrenal pedicles. This interpretation was supported by the observation that briefly clamping the adrenal pedicles bilaterally produced the same effect on hypothalamic CRF content as bilateral adrenalectomy. To pursue these findings, and to examine their physiological implications, we studied the effects of unilateral adrenalectomy or of manipulating the pedicle of one adrenal gland on the weight and composition of the contralateral gland.

If manipulation of the adrenal pedicle initiates a signal that is equivalent to adrenalectomy, then study of the response to adrenal manipulation would allow us to demonstrate clearly that removal of adrenal tissue is not required to initiate the compensatory adrenal response; this may give us a clearer understanding of the nature of the afferent signal. To conclude that the signals to growth are the same after adrenalectomy and adrenal manipulation, the growth must be shown to be of the same nature in both groups. Finally, if compensatory adrenal growth is mediated neurally rather than hormonally, then it should be possible to demonstrate growth of only one adrenal after manipulation in animals bearing two adrenals.

B. MATERIALS AND METHODS

Young male Sprague-Dawley rats (Simonsen, supplier) 100-150 g b.w. were received 1-4 d before use. Female rats 105-120 g were used in one experiment as indicated in the text. The rats were housed 2-3/cage and in a temperature and light controlled room (lights on 0600-1800 h) and were allowed rat chow and water ad libitum. In some experiments rats were treated before use with dexamethasone (Decadron, Merck, Sharpe and Dohme) 25 μg/100 g body weight subcutaneously to prevent ACTH secretion in response to the operation. Since the regimen of dexamethasone treatment varied, each experimental protocol will be delineated below.

Rats were anesthetized with ether and the left or right adrenal gland was exposed through a retroperitoneal incision. Adrenal manipulation consisted of gently bringing the gland to the skin surface by its pedicle. The pedicle was clamped with smooth forceps for a few sec until the gland blanched peripherally and flushed centrally, indicating alterations in arterial and venous blood flow; the pedicle was then released and the gland was returned to the abdominal cavity. Surgical sham rats were subjected to the same anesthesia and skin and muscle incisions; however, the adrenal gland was exposed but not touched. Unilateral adrenalectomy consisted of removal of one adrenal through similar incisions. The skin incisions were clipped and rats were returned to their home cages.

Retained blood in the adrenals was estimated using ^{51}Cr labeled rat red blood cells. The red cells were prepared by washing packed cells with acid-citrate-dextrose (ACD) and then incubating equal volumes of red cells and ACD with ^{51}Cr (New England Nuclear) in a shaking water bath at 37°C for 1 h. After the incubation the cells were washed thoroughly with ACD and diluted 1:3 with ACD for injection. Rats were anesthetized with ether, the red cells (11.3×10^6 cpm ^{51}cr in 0.2 ml) were injected through a tail vein and 3 min later the animals were decapitated. Adrenal glands and duplicate aliquots of blood were counted. At least 10,000 counts were collected. The calculated blood volume from the isotope injected was 7.40 ± 0.54 (S.D.) ml/100 g body weight (n=11).

Rats were killed by decapitation at the time indicated below and trunk blood was collected in chilled plastic centrifuge tubes for subsequent plasma ACTH (Dallman *et al.*, 1974; Grizzle *et al.*, 1974; Rees, *et al.*, 1971) and corticosterone (Murphy, 1967) analyses. Adrenals were removed and placed on ice in closed dishes on paper saturated with 0.9% NaCl. The chilled glands were cleaned of fat and connective tissue and weighed to the nearest 0.01 mg. In some experiments adrenals were dried in an oven to constant weight (3 weighings).

DNA and RNA were extracted from adrenal homogenates (1:100 w/v in distilled water) by the method of Schmidt and Thannhauser (1945) incorporating modifications recommended by Munro and Fleck (1966).

Protein in 0.5 ml homogenate was precipitated by adding an equal volume of 10% trichloroacetic acid (TCA) followed by 2 washings of the precipitate with 5% TCA to remove acid soluble compounds. Lipids were removed by 2 washings with 95% ethanol saturated with NaAc followed by 2 washings with 95% ethanol saturated with NaAc:diethyl ether (3:1 v/v). The precipitate was dissolved in 0.5 ml 0.3 N KOH for at least 2 h and then RNA was extracted by adding 0.5 ml cold IN $HClO_4$. The supernatant was saved and combined with a further 0.5 ml wash with 0.5 N $HClO_4$. RNA was assayed by the orcinol method in 0.5 ml samples. DNA was extracted by heating the precipitate at 70°C in 1.0 ml 0.5 N $HClO_4$ for 20 min. A second wash of 0.5 ml was combined with the initial extract and 0.5 ml samples were measured in duplicate using the method of Burton (1968). Calf thymus DNA (Sigma) and D-ribose (Sigma) were used as standards for these determinations. Protein concentration was determined by the method of Lowry (1951) using human albumin as a standard.

Experiment I. Male rats were subjected to left surgical sham, adrenalectomy or adrenal manipulation under ether anesthesia at 0900 h. Groups of 8 animals were killed 12, 24, 48 and 72 hours later. Unoperated controls were included at 12 and 72 h. Plasma ACTH and corticosterone, and right adrenal weights were measured.

Experiment II. Male rats were subjected to one of the 3 adrenal operations at 2100 h and were killed at 0900 h. Plasma ACTH and corticosterone were measured as well as right adrenal wet weight, DNA, RNA and protein content (n=6/group).

Experiment III. Male rats were subjected to one of the 3 adrenal operations at 0900 h and killed 12 h later at 2100 h. Plasma ACTH and

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corticosterone were measured in 14 rats/group. Right adrenal wet weight was measured in 18 rats/group. Right adrenal dry weight was measured in 6 rats/group. Right adrenal DNA, RNA and protein content were measured in 6 rats/group. Four rats in each group were injected with ^{51}Cr labeled rat red blood cells i.v. under ether anesthesia 3 min before sacrifice to determine the quantity of residual blood in right adrenals.

Experiment IV. Male rats (8/group) were subjected to left adrenal manipulation at 0900 h and groups were killed 0, 12, 24 and 72 h later. Left and right adrenals were weighed.

Experiment V. Groups of 6 male rats were treated with dexamethasone 25 µg/100 g body weight 2 h before left surgical control, adrenalectomy or adrenal manipulation. Six hours postoperatively, the rats were anesthetized with sodium pentobarbital 5 mg/100 g body weight and injected with 4 ng (0.4 mU) synthetic ACTH (Cortrosyn, Organon) i.v. The rats were killed 15 min later and plasma corticosterone concentrations and right adrenal weights were measured.

Experiment VI. Male rats were subjected to the surgical control or to the manipulative operation first on the right side in the morning and then on the left side at night 12 h later. Separate groups of rats were subjected to the right sided operation only at night. All animals were killed 12 h later in the morning. Plasma ACTH and corticosterone were measured as well as left and right adrenal DNA and RNA contents (n=6 to 8/group).

Experiment VII. Male rats were treated with 25 µg/100 g body weight dexamethasone 18 and 2 h before the first adrenal manipulation and 10 h postoperatively. The design of the experiment was the same as that in VI except that ACTH secretion was prevented using this regimen of dexamethasone treatment (Sirett and Gibbs, 1969).

Experiment VIII. Female rats were subjected to bilateral surgical control operations or to bilateral adrenal manipulation. The rats were killed 12 h later at 2200 h and adrenal wet and dry weights were measured (n=8 to 10/group).

Significance of the differences between groups was analyzed using Student's unpaired *t*-test. Significance of differences between left and right adrenals in the same rats was determined using the paired *t*-test.

C. RESULTS

I. Changes in plasma ACTH and corticosterone, and right adrenal weight after left manipulation or adrenalectomy.

Fig. V-1 shows plasma ACTH and corticosterone concentrations and right adrenal weight during the first 3 d after the operations of left manipulation, adrenalectomy or surgical sham. The operations were performed in the morning. For reference, unoperated control values are included at 12 h and at 72 h. All three operated groups have elevated ACTH levels at 12 h although only the surgical sham and adrenalectomy groups are significantly elevated above unoperated controls ($p < 0.05$). ACTH levels decline over the next days and do not differ from each other or from control by 72 h. Corticosterone levels generally reflect the ACTH levels. Right adrenal weight is highly significantly elevated above the surgical shams in both adrenalectomy and manipulation groups at 12 h ($p < 0.001$).

Because increases in circulating ACTH result in increases in adrenal weight (Imrie *et al.*, 1965), and because the initial operations stimulate prolonged (12 h) increases in ACTH levels, increases in

Fig. V-1. Changes in circulating ACTH and corticosterone concentrations and in right adrenal wet weight with time after the operations of left surgical sham, manipulation or adrenalectomy. Unoperated control rats are included at 12 and 72 h. Each bar represents the mean of 8 rats, and the line indicates ± SEM.

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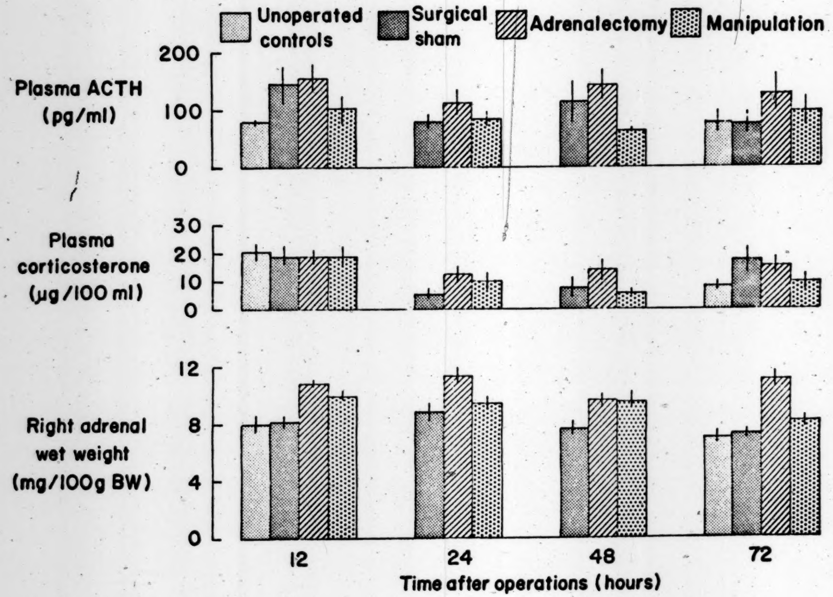
Unoperated controls
Surgical sham
Adrenalectomy
Manipulation
Plasma ACTH (pg/ml)
200
100
0
Plasma corticosterone (µg/100 ml)
30
20
10
0
Right adrenal wet weight (mg/100g BW)
12
8
4
0
12
24
48
72
Time after operations (hours)

adrenal weight in the experimental groups must be tested against the adrenal weights of surgical adrenal weights of surgical shams. Typically, adrenal weight of rats undergoing surgical sham adrenalectomy is greater at 24 h than it was pre-operatively, or than the adrenal weight of unoperated rats killed at the same time.

Compared to the surgical sham group, the increase in adrenal weight in the adrenalectomy group is sustained throughout the 3 day period ($p < 0.01$). In contrast, after left adrenal manipulation, the increased right adrenal weight observed at 12 h is not maintained; there is a fall in weight at 24 h and a subsequent increase ($p < 0.01$) at 48 h and then a second decrease at 72 h. In a previous experiment we also found a significant increase in right adrenal weight at 12 and 72 h after left adrenal manipulation compared to surgical sham ($p < 0.05$).

II. Changes in right adrenal nucleic acid and protein content after left adrenalectomy or manipulation.

To assure ourselves that the increase in right adrenal weight at 12 h after left adrenalectomy or manipulation was an effect primarily of the operation and was not dependent on the time of day, we repeated the 12 h experiment, doing the operations at night and killing the rats in the morning. Table V-1 shows the results of this experiment. We also measured nucleic acid and protein content in the right adrenals in an attempt to distinguish between the operations of adrenalectomy and manipulation. Comparison of the results in the table with those in Fig. V-1 shows that the changes in ACTH and right adrenal weight that occur after left manipulation or adrenalectomy are probably independent of the time of day at which the operations

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Table V-1

Effect of left surgical sham operation, adrenalectomy or manipulation performed at 2100 h on plasma ACTH and corticosterone concentration, and right adrenal weight, DNA, RNA and protein content 12 h later (n=6/group).

	Control	Surgical sham	Adrenalectomy	Manipulation
plasma ACTH pg/ml	$43\pm3^{\prime *}$	119±26	189±49	$52\pm3^{*}$
plasma corticosterone µg/100 ml	$2.0\pm0.2^{*}$	7.7±1.3	10.4±1.5	$2.7\pm0.3^{*}$
adrenal weight mg/100 g b.w.	$7.52\pm0.33^{*}$	8.62±0.30	9.90±0.61	10.21±0.61
DNA µg/100 g b.w.	44.8±2.6	47.9±3.8	$63.4\pm3.1^{*}$	$60.1\pm4.6^{*}$
RNA µg/100 g b.w.	47.1±2.5	51.6±3.5	$74.0\pm3.6^{*}$	$71.3\pm4.9^{*}$
protein mg/100 g b.w.	1.60±0.09	1.60±0.04	1.89±0.15	$1.91\pm0.08^{*}$

$^{\prime}$numbers represent mean ± 1 SEM

*different from surgical shams ($p < 0.05$)

are performed. Circulating ACTH and corticosterone levels were not different in the surgical sham and adrenalectomy groups whereas they were significantly depressed in the adrenal manipulation group ($p < 0.05$). Right adrenal weight was increased but not significantly ($0.05 < p < 0.1$) in both adrenalectomy and manipulation groups compared to surgical sham. The increases observed in the adrenal nucleic acid and protein content of the two experimental groups were more striking. DNA and RNA content were significantly elevated in both groups ($p < 0.01$) and protein content was increased in the manipulation group ($p < 0.05$).

III. Left adrenal dry weight, nucleic acid and protein content, residual blood content 12 h after the 3 operations.

Twelve hours is an exceedingly short time for such pronounced changes in adrenal DNA content to occur (Imrie *et al.*, 1965). In addition, although the increases in adrenal wet weight are on the order of 20-30%, the absolute magnitude of the differences in weight is small (a few mg.). If the mechanism involved in the increased adrenal weight includes hyperemia, then it seemed possible to us that part of the increase in adrenal weight observed at 12 h might result from an increased amount of blood retained by the glands post mortem. Therefore, we repeated the experiment, operating in the morning and killing the animals at night, and measured retained blood, dry weight, nucleic acid and protein content of the adrenals. Right adrenal wet weight and protein content were significantly increased in the two experimental groups compared to the surgical sham group ($p < 0.05$) (Figure V-2). DNA and RNA content were also significantly increased

Fig. V-2. Right adrenal wet weight, DNA, RNA and protein content 12 h after the operations of left surgical sham, adrenalectomy, or manipulation. Each bar represents the mean of 6 rats, and the line indicates ± SEM.

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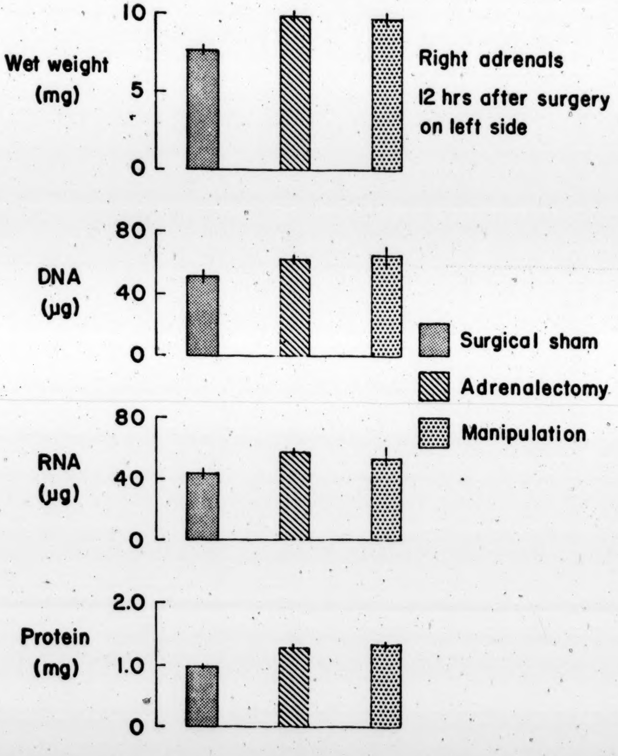
10
Wet weight
(mg)
5
0
Right adrenals
12 hrs after surgery
on left side
80
DNA
(µg)
40
0
Surgical sham
Adrenalectomy
Manipulation
80
RNA
(µg)
40
0
2.0
Protein
(mg)
1.0
0

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in the adrenalectomy ($p < 0.01$) but not in the manipulated group because of the large variance in this experiment. Adrenal dry weight was increased in the two experimental groups ($p < 0.05$), and there was no difference in residual blood per gland as measured by the ^{51}Cr tagged red cell content (Table V-2). Therefore, the increase in adrenal wet weight observed at 12 h after adrenalectomy or adrenal manipulation cannot be accounted for by increased water content or by an increased amount of residual blood in the glands. There was no significant difference in the circulating ACTH and corticosterone levels in the first 6 animals of each group decapitated; however, as the experiment progressed and successive groups of 6 rats were killed, a significant increase in ACTH occurred in the adrenalectomized and adrenal manipulated groups ($p < 0.05$), as though these animals were particularly responsive to the environmental stimuli associated with continued experimentation in the animal room. In summary, no parameter that we have measured, with the possible exception of circulating ACTH levels, has allowed us to distinguish between the operations of unilateral adrenalectomy and adrenal manipulation.

IV. Changes in left and right adrenal weight after left manipulation.

We next measured left and right adrenal wet weight 12, 24 and 72 h after left adrenal manipulation. Left adrenal weight is unchanged during the 3 d (Figure V-3); however, as usual, right adrenal weight is significantly increased at 12 h. Right adrenal weight falls at 24 h and tends to increase somewhat again at 72 h although the difference is not significant ($0.1 > p > 0.05$).

Table V-2 *Adrenal dry weight, residual blood, and plasma ACTH and corticosterone concentrations of rats 12 h after left surgical sham, adrenalectomy, or manipulation in the morning*

	Surgical Sham	Adrenalectomy	Manipulation
Right adrenal dry wt, mg	1.728 ± 0.142 (6)[*]	2.159 ± 0.103 (6)	2.073 ± 0.046 (6)
***P* vs. sham**		<0.05	<0.05
Right adrenal residual blood μl/gland	0.165 ± 0.011 (3)	0.136 ± 0.022 (4)	0.190 ± 0.030 (4)
***P* vs. sham**		NS	NS
μl/mg wet wt	0.0213 ± 0.0012 (5)	0.0140 ± 0.0018 (4)	0.0205 ± 0.0035 (4)
***P* vs. sham**		<0.05	NS
Plasma ACTH, pg/ml first 6	66 ± 9 (6)	76 ± 10 (6)	68 ± 6 (6)
***P* vs. sham**		NS	NS
Last 8	48 ± 5 (8)	138 ± 27 (8)	135 ± 40 (8)
***P* vs. sham**		<0.01	<0.05
Plasma corticosterone first 6	14.8 ± 2.7 (6)	9.9 ± 1.4 (6)	12.6 ± 2.4 (6)
***P* vs. sham**		NS	NS
Last 8	14.1 ± 1.9 (8)	16.3 ± 1.8 (8)	15.9 ± 2.3 (8)
***P* vs. sham**		NS	NS

[*] **Numbers are means ± 1 SE; the numbers in parentheses are the numbers of rats in each group.**

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Fig. V-3. Left and right adrenal wet weight from rats subjected to left adrenal manipulation at time 0. Each point represents the mean adrenal weight of at least 6 rats. The difference between left and right adrenal weight at 12 h was significant at the 1% level (paired _t_ test).

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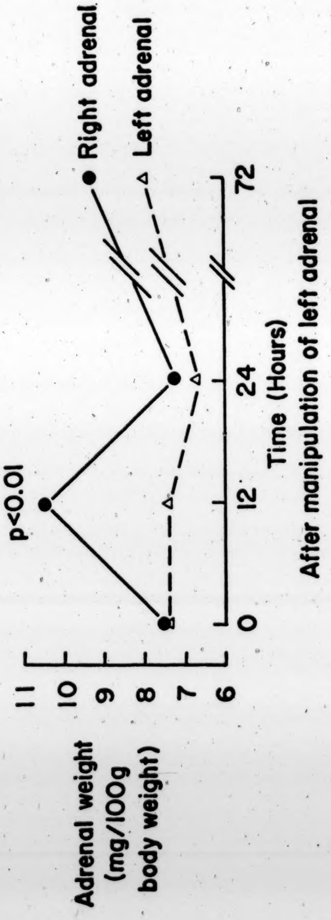
Adrenal weight
(mg/100g
body weight)
11
10
9
8
7
6
0
12
24
72
Time (Hours)
After manipulation of left adrenal
p<0.01
Right adrenal
Left adrenal

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V. Plasma corticosterone response to exogenous ACTH 6 h after left adrenal manipulation or adrenalectomy.

We have no evidence that adrenal manipulation inhibits the ability of the manipulated adrenal to respond to ACTH; however, adrenal manipulation could impair adrenal function. In the initial experiment comparing plasma ACTH and corticosterone responses to the 3 operations (not shown), the corticosterone response of the manipulated groups was never significantly less than that of the surgical sham animals at 6 times during the first 4 h, although there was a transient deficit in the corticosterone response of unilaterally adrenalectomized rats (Chapter II). We tested the capacity of all three groups to respond to exogenously administered ACTH 6 h after operations performed in dexamethasone pretreated rats. Adrenal manipulation did not impair the plasma corticosterone response to ACTH but left adrenalectomy did ($p < 0.01$) (Table V-3).

The results of the acute response to ACTH in the three groups indicate that both the manipulated and contralateral adrenal are capable of receiving the ACTH signal and of secreting corticosterone, whereas only the gland opposite to manipulation increases in size at 12 h. These results suggest either that the growth response is neurally mediated or that the manipulated gland is damaged subtly so that although it can respond to ACTH it is incapable of responding to another humoral agent that promotes growth.

VI and VII. Sequential adrenal manipulations at 12 h intervals.

To test whether the manipulated adrenal was itself capable of the growth response, we performed two experiments. In the first, rats

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Table V-3

Secretory response of adrenals after left manipulation, left adrenalectomy or surgical control operation. All rats were treated with dexamethasone (25 µg/100 g b.w.) 2 h preoperatively. At 6 h postoperatively, the rats were anesthetized with ether and given 0.4 mU ACTH i.v. Plasma corticosterone and adrenal weight were measured at 15 min. (n=6/group).

	Plasma corticosterone µg/100 ml	Adrenal wet weight mg/100 g b.w.	
		left	right
surgical sham	30.8±2.7[*]	9.50±0.65	9.60±0.35
adrenalectomy	13.7±4.8[**]	----	10.81±0.30
manipulation	28.1±1.6	9.98±0.59	10.20±0.90

[*] numbers represent means ± 1 SEM

[**] significantly lower than surgical shams $p < 0.01$

were subjected to right surgical sham or manipulation at 0 time, and then at 12 h the rats were subjected to left surgical sham or manipulation. Additional groups of animals were exposed to right adrenal surgical sham or manipulation at 12 h. All rats were killed at 24 h. Adrenals were weighed and analyzed for DNA and RNA content. Whereas an increase in adrenal weight was observed in the surgical controls between 12 and 24 h, the increment on the left side was comparable to that on the right. There was no difference between left and right adrenal weight at 12 or 24 h in this group, and there was no difference between left and right adrenal DNA content at 12 h. At 24 h, there was a highly significant increase in DNA in the left compared to the right adrenal of rats that had undergone the left surgical sham operation 12 h previously. We do not know the cause for the increase in left adrenal DNA in this group, however, the response is opposite that observed in the manipulation group. In contrast to the responses observed in the surgical shams, 12 h after right manipulation, the left adrenal weight and DNA content were increased relative to the right adrenal or to the left adrenal of the surgical shams ($p < 0.05$). At 24 h, 12 h after left manipulation the opposite gland was increased in weight and DNA content ($p < 0.05$).

We repeated the experiment on rats that were pretreated 18 and 2 h before the first manipulation as well as 10 h later with dexamethasone, 25 μg/100 g body weight, to prevent stress-induced ACTH secretion and subsequent adrenal growth resulting from the action of ACTH. The results of this experiment are shown in the third row in Table V-4 (Experiment II). Dexamethasone treatment resulted in small adrenals

Table V-4 *Adrenal wet weight, DNA, and RNA content after sequential adrenal manipulation applied at 12-h intervals*

	Weight, mg/100 g body wt		DNA, μg/100 g body wt		RNA, μg/100 g body wt	
	12 h	24 h	12 h	24 h	12 h	24 h
Group 1A) Surgical sham Right side at 0 h ($n = 8$) Left side at 12 h ($n = 8$)						
Left adrenal	8.69 ± 0.51	10.29 ± 0.83	50.8 ± 3.5	59.1 ± 1.6‡§	55.4 ± 3.3	64.4 ± 5.1
Right adrenal	8.51 ± 0.47	9.81 ± 0.72	53.4 ± 3.6	52.6 ± 2.4	57.8 ± 4.3	59.9 ± 4.5
Group IB) Adrenal manipulation Right side at 0 h ($n = 8$) Left side at 12 h ($n = 6$)						
Left adrenal	10.35 ± 0.59*†	11.07 ± 0.61	56.6 ± 1.6*†	55.8 ± 3.4	66.8 ± 3.5*	63.8 ± 3.6
Right adrenal	9.08 ± 0.47	11.86 ± 0.52*	51.5 ± 2.0	71.0 ± 7.2*†	62.9 ± 4.2	75.9 ± 3.5
Group II) Adrenal manipulation in dexamethasone-treated rats Left side at 0 h ($n = 9$) Right side at 12 h ($n = 7$)						
Left adrenal	6.74 ± 0.31	8.16 ± 0.62†	41.2 ± 1.9	52.3 ± 4.5	33.5 ± 2.0	36.7 ± 3.2
Right adrenal	8.01 ± 0.62‡	6.90 ± 0.47	53.3 ± 3.2‡	46.0 ± 3.4	40.2 ± 2.3‡	32.7 ± 1.5

Values are means ± SE. If adrenal manipulation is performed first on one gland, and at 12 h on the other gland, the first manipulated adrenal can respond at 24 h. * $P < 0.05$ between adrenal manipulation and surgical control, same adrenal comparison (unpaired-*t* test). † $P < 0.05$, left vs. right adrenal (paired-*t* test. ‡ $P < 0.01$, left vs. right adrenal (paired-*t* test). § Although the difference between right and left adrenals is significant, the increase occurs in the last observed adrenal, and is, therefore, opposite to the results obtained after adrenal manipulation.

on the most recently manipulated side but did not inhibit the normal increase in adrenal weight of the contralateral gland ($p < 0.05$). The results clearly show that after manipulation of one adrenal, both are capable of responding, and therefore, strongly suggest that the unilateral response is neurally mediated.

VIII. Response to simultaneous bilateral adrenal manipulation.

Finally to assure ourselves that there was not a transient period of adrenal unresponsiveness to a blood borne agent that promotes adrenal growth, we compared the results of bilateral surgical sham operation and bilateral adrenal manipulation in female rats on adrenal wet and dry weight 12 h later. After bilateral adrenal manipulation both the left and right adrenal weights were consistently increased compared to those of the bilateral surgical shams ($p < 0.01$ in each comparison) (Table V-5), thus showing that manipulation does not impair the ability of the manipulated adrenal to grow.

D. DISCUSSION

In this study we have demonstrated that the operations of left adrenal manipulation or left adrenalectomy produce equivalent responses in the right adrenal 12 h later and that the responses are not dependent on the time of day the operations are performed or the time the animals are killed. The response of the contralateral gland consists of an increase on the order of 20-30% in wet weight, dry weight, DNA, RNA, and, usually, protein content. The volume of blood retained in the right adrenal after left adrenalectomy or manipulation is not greater than that in surgical sham groups. The response is not dependent on elevated circulating ACTH levels since we have shown

Table V-5

Bilateral adrenal manipulation results in bilateral adrenal enlargement 12 h later in female rats.

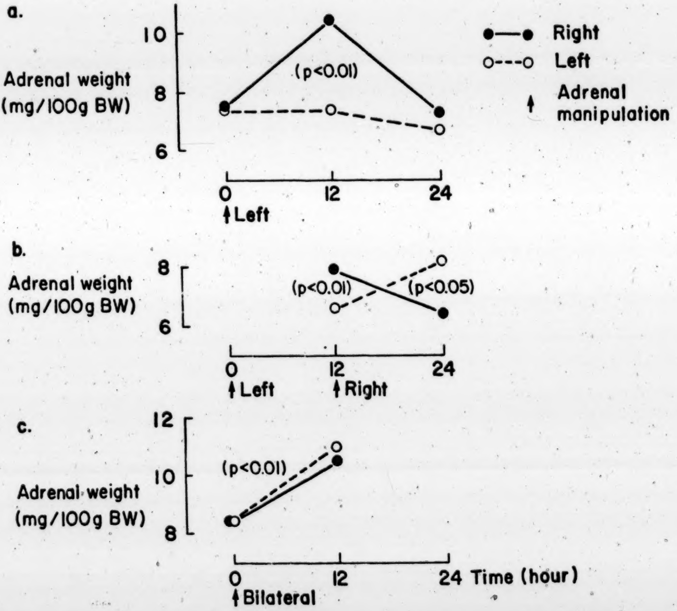
		Bilateral		
		Surgical sham (n=8)	p	Manipulation (n=12)
Adrenal wet weight mg/100 g b.w.	left	8.08±0.51	<0.005	11.05±0.35
	right	8.12±0.55	<0.01	10.59±0.26
Adrenal dry weight mg/100 g b.w.	left	2.775±0.184	<0.005	3.670±0.095
	right	2.806±0.205	<0.005	3.568±0.110

previously that it occurs in hypophysectomized rats after adrenalectomy (Chapter II) and in this study that it occurs after adrenal manipulation in rats pretreated with dexamethasone using a regimen known to inhibit ACTH secretion (Chapter II; Sirett and Gibbs, 1969).

Because we have found no measure that distinguishes at 12 h between the increase in right adrenal weight after left adrenalectomy and that which occurs after adrenal manipulation, we assume that clamping the vascular and nervous supply of one adrenal may initiate signals that are similar to those that result from unilateral adrenalectomy. Because adrenal manipulation results only in transient effects, we assume that we have reversibly inhibited or stimulated nerves conveying information from the adrenal gland to the central nervous system.

The results of these experiments suggest strongly that manipulation of one adrenal stimulates or inhibits afferent nerves and that this stimulation or inhibition results in initiation of the compensatory growth response in the contralateral gland. Fig. V-4 summarizes the experimental results from this study that suggest this conclusion. It is clear that the contralateral gland enlarges when one adrenal is manipulated, that the glands enlarge sequentially after sequential manipulation, and that both will enlarge if they are both manipulated. Since a blood-borne humoral factor could not account for these findings, the results necessitate the interpretation that growth of the opposite gland is initiated by a neural mechanism.

Fig. V-4. Evidence that the reflex is neurally mediated. Panel a shows the results from Fig. V-3; panel b shows the results from Table V-4, experiment II; and panel c shows results from Table V-5.


a.
Adrenal weight (mg/100g BW)
6
8
10
(p<0.01)
0
12
24
Left
●—● Right
○---○ Left
Adrenal manipulation
b.
Adrenal weight (mg/100g BW)
6
8
(p<0.01)
(p<0.05)
0
12
24
Left
Right
c.
Adrenal weight (mg/100g BW)
8
10
12
(p<0.01)
0
12
24 Time (hour)
Bilateral

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CHAPTER VI

CONCLUSION

The classical explanation for compensatory adrenal growth after unilateral adrenalectomy has been that removal of one adrenal results in a hypocorticoid signal which decreases the feedback inhibition of ACTH secretion. The resulting high levels of ACTH would stimulate the growth of the remaining adrenal (Collip _et al_., 1932). In the present studies, no evidence was obtained supporting this hypothesis. In dexamethasone-treated animals and in hypophysectomized animals, compensatory growth was observed after unilateral adrenalectomy (Chapter II). Thus, neither a hypocorticoid signal nor the hypersecretion of ACTH or any other pituitary hormone was required for compensatory adrenal growth. The increases in adrenal weight after unilateral adrenalectomy were characterized by increases in DNA content by 12 hours followed by increases in RNA and protein content (Chapter III; Chapter V). Since increased DNA content indicated the occurrence of cell proliferation, the weight increase after unilateral adrenalectomy has been referred to in the present studies as compensatory adrenal growth, not compensatory adrenal hypertrophy. Whereas ACTH stimulates adrenal hypertrophy by increasing adrenal RNA and protein content, the presence of circulating ACTH was inhibitory to the DNA content increases observed after unilateral adrenalectomy (Chapter III). The term "compensatory adrenal hypertrophy" strongly implies that ACTH is responsible for the weight increases after unilateral adrenalectomy; given the data presented in earlier chapters, "compensatory adrenal growth" or "compensatory adrenal hyperplasia" seem more appropriate to describe the adrenal weight increase after unilateral adrenalectomy. Compensatory growth was blocked by unilateral lesions in the hypothalamus and by hemi-sections of the spinal cord; treatment of the adrenal pedicle with a local anesthetic also prevented compensatory growth (Chapter IV). The design of these experiments suggested that both the afferent and efferent components of the compensatory response were neural. Additional support for this hypothesis was provided by adrenal manipulation experiments in which one adrenal was stimulated to grow in the presence of both adrenals (Chapter V). Together, these data demonstrate that compensatory adrenal growth after unilateral adrenalectomy is mediated by a neural reflex composed of afferent and efferent neural components and that the hypothalamus acts to monitor adrenal size.

Study of the effects of ACTH on the response to unilateral adrenalectomy led to the unexpected finding that the presence of ACTH delays the development of compensatory adrenal growth. Treatment with dexamethasone which blocks ACTH secretion accelerates the occurrence of increased adrenal weight and DNA content after unilateral adrenalectomy. In addition, treatment of hypophysectomized rats with ACTH at the time of unilateral adrenalectomy inhibits compensatory growth 3 days later. These data are in close agreement with results obtained using cultured, functional mouse adrenal tumor cells (Masui and Garren,1971). In this work, ACTH treatment increased steroidogenesis but inhibited thymidine incorporation into adrenal DNA and blocked the increase in DNA content normally observed in these rapidly

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dividing cells. Another report using a different adrenal tumor cell line confirmed the finding that ACTH can inhibit adrenal DNA synthesis (Weidman *et al*., 1976). Cultured, Y-1 adrenal tumor cells were stopped in the G-1 phase of the cell cycle following ACTH administration; the cells, which did not synthesize DNA, grew in size and synthesized RNA and protein in response to ACTH. While it is difficult to draw comparisons between *in vivo* and *in vitro* adrenal responses, it appears that the growth response after unilateral adrenalectomy and adrenal tumor growth have certain similarities. Both compensatory growth and tumor growth are characterized by rapid increases in DNA content and both are suppressed by ACTH administration. Thus, study of the mechanisms regulating compensatory adrenal growth may lead to a better understanding of the factors controlling tumor growth.

A major finding of the present studies was that ACTH is not responsible for compensatory adrenal growth after unilateral adrenalectomy. The data supporting this conclusion are outlined below:

a) Following treatments which removed endogenous sources of ACTH, such as dexamethasone administration and hypophysectomy, compensatory adrenal growth was still observed (Chapter II).

b) The initial adrenal weight increases stimulated by ACTH consist of increases in RNA and protein content. In contrast, the increases in adrenal weight 12 hours after unilateral adrenalectomy consist of increases in DNA content (Chapter III; Chapter V). Thus, the initial weight increases stimulated by ACTH are due to adrenal hypertrophy, whereas the compensatory response is adrenal hyperplasia.

c) Increases in adrenal DNA content occur following 3-7 days

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treatment with large amounts of ACTH (4-20 U) (Chapter III; Farese and Reddy, 1963; Masui and Garren, 1971). The increase in adrenal DNA content after unilateral adrenalectomy occurred by 12 hours and the circulating ACTH levels during the 12 hour period presumably were below those required to stimulate DNA synthesis (Chapter II).

d) The development of compensatory adrenal growth was delayed by exogenous ACTH treatment and accelerated by removing endogenous ACTH with dexamethasone (Chapter III).

The work of Halasz and Szentagothai (1959) suggested that unilateral adrenalectomy could alter the activity of neurons on one side of the hypothalamus differently from neurons on the opposite side of the hypothalamus. This unilateral effect led us to investigate the role of the nervous system in compensatory adrenal growth. The finding that unilateral lesions at hypothalamic and spinal cord levels blocked compensatory growth (Chapter IV) supports the hypothesis that growth after unilateral adrenalectomy is neurally mediated. These results suggested that the efferent component of the adrenal response is mediated via a crossed neural pathway originating in or passing through the hypothalamus and descending via the spinal cord to the adrenal gland.

The suggestion that neural activity can stimulate adrenal growth is supported by earlier work in which neural activity has been implicated to explain tissue growth (Brenner and Stanton, 1970; Thrower _et_ _al_., 1973; Ashrif _et_ _al_., 1974; Muir _et_ _al_., 1975). Electrical stimulation of sympathetic fibers shown to innervate the submaxillary and parotid salivary glands increased the wet weight, mitotic index and thymidine incorporation of these exocrine glands.

This growth, which could be blocked by propranolol and increased by isoproterenol, was ascribed to stimulation via a β-adrenergic receptor mechanism (Muir _et al_., 1975). In addition, liver regeneration after partial hepatectomy can be neurally influenced. Following liver denervation or treatment with reserpine or propranolol, the rate of liver regeneration determined by incorporation of thymidine into liver DNA is significantly suppressed (Ashrif _et al_., 1974). Thus, alteration of normal neural activity by pharmacological blockade or surgical intervention can affect subsequent tissue growth. The physiological significance of the neural control of compensatory adrenal growth cannot be estimated at this time; while clinical examples of adrenal hyperplasia without increased levels of ACTH are well-documented (Liddle and Melmon, 1974), a role for neural stimulation in these cases has not been investigated.

While the afferent component of the compensatory response may travel with the efferent component via the spinal cord, the anatomical definition of this pathway remains unclear. However, direct evidence for the neural nature of the afferent component was found in the lidocaine experiment. Treatment of the adrenal gland before its removal with a local anesthetic inhibited compensatory growth (Chapter IV). Additional support for the hypothesis that both afferent and efferent components are neural was provided by experiments in which growth was stimulated unilaterally in the presence of both adrenals (Chapter V). The signal provided by manipulating one adrenal had to be neural since the response to the signal was unilateral; that is, a hormonal signal would provide afferent stimulation to the efferent component to both adrenals and the resultant response would be bilateral. Thus, a neural reflex composed of afferent and efferent components is responsible for compensatory growth after unilateral adrenalectomy.

The physiological significance of a neural pathway which influences adrenal growth can only be determined with further experimentation. However, two considerations are immediately brought to mind. First, the finding of neural influences on the adrenal cortex implies that a physiological control system in addition to ACTH may drive the adrenal. This possibility is supported by work on the thyroid gland and endocrine pancreas in which hormonal and neural activity have been shown to control organ function. The secretion of thyroid hormones, stimulated by thyroid-stimulating hormone (TSH) released from the pituitary, is influenced by stimulation of the thyroid nerve supply. Following blockade of TSH by exogenously administered thyroxine, unilateral electrical stimulation of the cervical sympathetic trunk induced increased formation of intracellular colloid droplets within the thyroid gland and an increase in plasma thyroid hormone levels (Melander _et al_., 1972). The droplet formation, which was restricted to the thyroid regions supplied by the stimulated nerve, was inhibited by phentolamine (an alpha-adrenergic receptor blocker). With the ultrastructural identification of noradrenergic nerve endings in close contact with thyroid follicular cells (Melander _et al_., 1974), it was suggested that alpha-adrenergic innervation of the thyroid could serve as a means for effecting alterations in the rate of thyroid hormone secretion. In the endocrine pancreas, both adrenergic and cholinergic mechanisms are known to affect insulin secretion (Woods and Porte, 1974). Alpha-adrenergic stimulation was shown to decrease basal plasma insulin levels, whereas beta-adrenergic stimulation increased basal levels. The authors suggested that basal pancreatic β cell secretion might reflect the balanced effects of adrenergic stimulation. While removing parasympathetic pancreatic fibers by vagotomy had no effect on basal plasma insulin levels, glucose tolerance was decreased. In addition, plasma insulin levels were increased by administering cholinergic agonists or by stimulating the vagus nerve. It was suggested that a cholinergic mechanism might act at the level of the β cell to potentiate insulin release in response to blood glucose stimulation. In the adrenocortical system, future research must delineate how neural activity affects adrenocortical function and how ACTH and neural input interact to control the adrenal gland.

Second, compensatory growth has been reported for a variety of tissues, including ovary, testes, thyroid, liver, salivary gland and kidney (Bucher and Malt, 1971); however, no clear identification of the signals initiating this growth has been found. Since neural activity has been shown to stimulate growth in some of these tissues (i.e., salivary gland and liver), it is possible that neural mediation of compensatory growth is a general phenomenon. The existence of separate neural pathways involved in monitoring and controlling specific tissue growth might be established by future investigation.

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